

**Associations and Movement Patterns of Reproductive Female
Wolverines (*Gulo gulo luscus*) on the Southeast Alaska Mainland**



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INTRODUCTION

Despite significant gains in our understanding of wolverine ecology and habitat associations in North America over the last 3 decades (Hornocker 1981, Magoun 1985, Gardner 1985, Banci 1987, Copeland 1996, Persson 2003, Persson et al. 2009, and others), a major gap exists in our knowledge of wolverines inhabiting coastal ecological zones. For example, in Southeast Alaska (SE AK) the only information available on wolverines is from Alaska Department of Fish and Game (ADFG) sealing records of animals taken during the hunting and trapping season. These records only contain information on hunter or trapper identification, month/year of harvest, location and method of harvest, method of transportation, and sex of the animal. During the 10-yr period 1994–2003, trappers and hunters harvested an average of 24 wolverines annually in SE AK, with Game Management Unit (GMU) 1B producing an average of 7 (range 1–18) wolverines per year. Sealing records indicate that GMU 1B and 1C (Fig. 1), where we conducted field work for this study, typically produces the highest wolverine harvest in SE AK.

The large expanse of remote, mountainous habitat in SE AK has provided wolverines with expansive refugia from trapping, which along with immigration from Canada has probably helped sustain viable populations in the region. However, recent genetic studies indicate that there may be little interchange between SE AK wolverine populations and those in adjacent Canadian provinces, particularly the female segments of the populations (Frances 2008). Further genetic studies are needed to determine the extent of dispersal between these adjacent populations (Tomasik and Cook 2005). Functional refugia are important in maintaining source populations to repopulate areas where trapping occurs (Magoun and Copeland 1998, Krebs et al. 2004, Golden et al. 2007). Refugia must be large enough and contain enough suitable habitat for reproductive females to successfully den and rear kits without having to make excursions into trapped areas. There is, however, no information currently available on denning habitat, home range size, movements, and the extent or frequency of extra-territorial movements for reproductive female wolverines in SE AK.

A key component of viable wolverine populations is the survival of reproductive females (Persson 2003). Although mature female wolverines have the capacity to produce

2-3 young per year, studies have shown that the actual reproductive rate for wolverines is much lower than expected, primarily due to delayed maturity and frequent failure to recruit young in years following successful reproduction (Persson 2005). Estimates of logistic growth rate for trapped populations of wolverines indicated that wolverine populations would decline in the absence of immigration from untrapped populations (Krebs et al. 2004). Population viability analyses of wolverines in Sweden indicated that for populations to remain viable, according to the International Union for Conservation of Nature and Natural Resources (IUCN) classification criteria (IUCN 2000), the number of sexually mature females (≥ 3 years old) should exceed 46 individuals (Persson 2003). There are currently no population estimates for wolverines in SE AK and no information on age or reproductive status of animals harvested in the region.

Resource development, human settlements, and increased human access in wolverine habitat can have negative impacts on wolverine populations, especially where dispersal potential for wolverines is compromised by disjunct habitats or lack of refugia (Weaver et al. 1996). Landscape-scale development activities in wolverine habitat such as those resulting from forest management activities not only modify habitat characteristics but affect wolverine movements (Bowman et al. submitted). Road construction associated with forest management activities and other development can increase trapper access to previously untrapped areas that once functioned as refugia for reproductive segments of a wolverine population (Dawson et al. submitted). Roads also provide travel corridors for wolves, which could increase the potential for mortality of denning females and their kits (Magoun and Copeland 1998).

Until 2007 when this study and another near Juneau (S. Lewis, unpublished data) were initiated in SE AK, there had been no studies of wolverines in coastal ecosystems, largely due to the expense and inherent difficulties associated with studying the species in these ecosystems. Wolverine populations are notoriously difficult to study because of the remoteness of their habitats, the size of their home ranges, and their low density compared to other mid-size carnivores. Acquiring information on reproductive females is particularly difficult because their home ranges often incorporate some of the most remote areas of occupied wolverine habitat. We conducted a pilot study in winter 2006–2007 to determine which portions of the GMU 1B Mainland most likely supported

reproductive female wolverines and what methods were most suitable for documenting wolverine movements and habitat associations in SE AK (Magoun et al. 2007). In late winter 2008 we conducted both a telemetry study and a camera-trapping study of wolverines, expanding our research objectives to include developing and implementing a method for estimating population density (Magoun et al. 2008), and in late winter 2009 we integrated our camera-trapping with hair snagging for DNA analysis of wolverines visiting the camera sites to validate our photo identification technique (Magoun et al. submitted) and to estimate survival rates and proportion of reproductive females in the study area (ongoing).

STUDY AREA

The study area (Fig. 1) comprised 2,140 km² of coastal tidelands, temperate rainforest, muskeg, alpine habitats, and glaciers in the Tongass National Forest on the mainland of Southeast Alaska near Petersburg (56° 48.67602' N, -132° 57.07452' W). Boundaries of the study area were Port Houghton on the north, Le Conte Bay on the south, Frederick Sound on the west, and the Stikine Icefield on the east. Most forested habitat occurred within 20 km of the coast and comprised Sitka spruce (*Picea sitchensis*) or western hemlock (*Tsuga heterophylla*) on productive forest sites and mountain hemlock (*Tsuga mertensiana*), Alaska-yellow cedar (*Chamaecyparis nootkatensis*), or western red cedar (*Thuja plicata*) on poorly drained sites. Large mammal carcasses available as food for wolverines in winter included mountain goat (*Oreamnos americanus*), Sitka black-tailed deer (*Odocoileus hemionus*), and moose (*Alces alces*). Temperatures at sea level during the study ranged from -4° C to 7° C but were considerably colder at higher elevations, although rarely falling below -23° C. Snowfall during the study was very heavy, reaching depths >4 m at higher elevations.

The study area included portions of GMU 1B and 1C as well as portions of the Juneau, Petersburg, and Wrangell Ranger Districts (U. S. Forest Service; USFS). Human inhabitants and developments were clustered near Point Agassiz and residents of the area had limited access to the surrounding forest on a small, local network of old logging roads (Fig. 1). Access for trapping was either along this road system with snowmachines and 4-wheelers or along the coast with boats. The average annual harvest of wolverines

in the study area in 2000–2009 was 2 (range 1–6) with males comprising 67% of the total harvest for those years. Trappers limited their efforts to take wolverines in 2007–2009 in cooperation with this study.

GOALS AND OBJECTIVES

The goal of this research was to provide wildlife managers with the information needed to ensure that wolverine populations are managed sustainably and that functional habitat for reproductive female wolverines is maintained in the face of increasing human development and access to remote areas in SE AK.

The specific objectives for the study were as follows:

1. Provide information on wolverine movements, home range, and denning habitat in the study area.
2. Use digital motion-detection cameras to gather additional information on the distribution and numbers of wolverines in the study area.
3. Capture hair for DNA from photographed wolverines to test the effectiveness of photo-IDs in establishing the identity and sex of wolverines.
4. Derive a population estimate for wolverines in the study area in 2008.
5. Collect tissue samples from harvested wolverines across SE AK, as well as hair from livetrapped wolverines and wolverines visiting camera sites, for use in genetic analysis of population structure and interchange with adjacent regions.
6. Further evaluate and refine an integrated camera and hair snag (C&H) technique as a method for estimating wolverine distribution and population size as well as for monitoring wolverines over the long term.

METHODS

Livetrapping

In winters 2006–2007 and 2007–2008, we livetrapped wolverines using box traps (Lofroth et al. 2008) that were constructed of 4 inch x 6 inch (89 mm x 140 mm) rough hewn timbers and that were roughly 152 cm (60 in) long, 76 cm (30 in) wide, and 51 cm

(20 in) deep (Fig. 2). Wolverines triggered the heavy timber lid when they pulled at a piece of bait secured inside the trap at the back near the floor and connected by a cable to a panic snap on the outside of the trap. A cable extended from the front of the trap lid, over a cross member above the trap, to the back of the trap where it was attached to the panic snap and held the lid up until the wolverine tugged on the bait and released the panic snap. We lubricated the trigger mechanism with petroleum jelly and covered the trigger with a plastic bag to prevent ice build-up. We removed loose hide from pieces of deer and moose carcasses used as bait to prevent a wolverine from stripping lengths of hide from the bait, which could lengthen the distance between the bait and the back of the trap. This prevented the possibility of a wolverine pulling on the bait and triggering the trap while its head was up or its body was in the trap opening. We attached “chew” logs to the inside front of the traps where wolverines most often tried to chew out of the trap. These logs were easily replaced when they became worn. After having wolverines escape twice by chewing their way out of traps in 2007, we reinforced all traps with hemlock lumber inside the traps and, in some cases, plywood on the outside of the traps. These reinforcements, combined with using trap-monitoring devices, prevented any wolverines from escaping the traps in 2008. With the amount of snow and rain in the study area during 2007 and 2008, trap lids remained heavy enough (>45 kg) that the wolverines could not lift the lids and escape.

We checked traps daily at a maximum of every 8 hours, either by visually inspecting them or by monitoring VHF signals emitted by trap-monitoring transmitters (Telonics®, Inc., Mesa, AZ) attached to the traps. When possible, we inspected most traps equipped with trap monitors every second day to remove snow and to ensure the trigger mechanisms were functioning properly. During periods of heavy snow followed by warm weather and rapid thawing, we unset traps or checked them every day to prevent water from building up inside the traps. During periods when sea ice was forming in Thomas Bay, we closed traps that were accessed by boat. We also set digital motion-detection cameras (Trailwatcher®, Monticello, GA; Cuddeback®, Nontypical, Inc., Green Bay, WI; Stealth Cam®, Grand Prairie, TX; and Reconyx®, Holmen, WI) at the traps to determine when wolverines began to visit them. We often did not set a trap

unless we knew it was being visited by a wolverine to reduce the need to visit traps to release marten, the only other species that triggered the traps.

We anesthetized captured wolverines with tiletamine HCl and zolazepam HCl (Telazol®, Fort Dodge, IA) at a dosage of about 10 mg/kg administered with a 6-cc syringe and 18-gauge needle attached to a jabstick. While one person used a long stick to distract the wolverine, another person would slowly move the jabstick and syringe close to the injection site (shoulder or rump) and then quickly jab the animal to inject the drugs. Once immobilized, we weighed the animals, measured the head and neck circumferences, took blood samples, collected hair for DNA and stable isotope analysis, and attached a combination satellite/VHF collar or a VHF-only collar (Sirtrack®, Havelock North 4147, New Zealand).

The Sirtrack satellite/VHF collars used the Argos satellite system administered by CLS America, Inc. (Washington, D.C.). The collar material was either rolled leather or flexible, flat nylon with a width of ≤ 25 mm (Fig. 3). Once activated the Argos transmitters emitted signals around the clock (i.e., we did not program a duty cycle to extend the life of the collars). We downloaded locations at regular intervals (≤ 10 days) using the CLS America website and entered the relocations in a GIS database; we also purchased the data CDs from CLS America as backup. We radiotracked wolverines wearing both types of collar using a PA-18 Supercub. In addition to basic relocation data, we also collected information on habitat characteristics where animals were located during the aerial tracking flights.

We categorized females as parous (evidence of past litters or current pregnancy) or nulliparous (no evidence of past or current pregnancies) based on teat development: dark-colored teats ≥ 4 mm long indicated females that had produced kits in the past or were currently pregnant and teats that were small, light-colored, and hard to detect indicated nulliparous females (Fig. 4). While females in the first category could be considered adults (average age at production of first litter ≥ 3 years; Persson 2006), age determination for nulliparous females was not possible. Even females as old as 8 years that have not produced a litter in their lifetime have teats that indicate nulliparity as described above (A. Magoun, personal observations of captive wolverines). Although tooth wear has been used to indicate approximate age in wolverines (e.g., Magoun 1985),

more recent information from known-age, wild wolverines indicates that wolverines as old as 12 years may have teeth in very good condition that would normally be associated with young individuals (J. Persson, unpublished data), so we did not use tooth wear as an indication of age.

We used size of testes and bacula to estimate an age category for males, either ≤ 16 months or >16 months. An approximate age determination for male wolverines based on relative testes size is only possible in livetrapped or photographed individuals from late February through June (Mead et al. 1991). Based on information from captive animals, testes of 1-yr-old males in this period are noticeably smaller than those of older males (Figs. 5; C. Long, D. Pedersen, and S. Kroschel, unpublished data). In addition, for livetrapped males, palpating the baculum provided information on the relative maturity of males because bacula of yearling males are relatively short (<8 cm) and thin; therefore, an approximate age category for males livetrapped in their first winter can be estimated with this technique by researchers experienced in handling both age groups.

We also photographed the ventral side of the animals because individual wolverines can be identified by the ventral pattern of the light-colored pelage on their throat and chest, including the gular patch near the chin (Fig. 6). We photographed individual markings while the animal was positioned on its back and on each side, both before and after collaring the animal, and again while the animal was coming out of the anesthesia in a more upright position (Fig. 7). We could compare these photographs with those taken with motion-detection cameras or harvested wolverines, in the event that the animals were able to remove their collars.

Motion-detection Cameras

Besides using cameras to monitor activity at the live traps in 2007 and 2008, we also deployed Trailwatcher cameras at baited camera stations in 2008 and 2009 to photograph ventral patterns. At each station we attached a 120-cm-long run pole horizontally to a tree with the proximal end at least 1 m above bare ground or snow and with the distal end pointed toward another tree ~ 3 m from the first tree (Fig. 8). We fabricated the run pole from 2-inch x 4-inch dimensional lumber (38 mm x 89 mm) or used a natural log (~ 15 cm diam.) found on site. We attached a Trailwatcher camera to the tree opposite the run

pole so that the camera faced the distal end of the run pole. The camera was set to take photos with the flash on both day and night. The camera triggered every time an animal moved in front of the camera, resulting in photos taken ~4 seconds apart for 500-700 photos on a 2 gigabyte memory card. For back-up in case of malfunction, battery depletion, or a full memory card, we also attached another Trailwatcher camera or an infrared Reconyx camera (no flash) to the tree and set them to take photos over a longer period of time. The Reconyx cameras generally produced poorer quality photos but would operate at temperatures below -23° C. We adjusted the height and position of the cameras so that the identification number on the end of the run pole would be centered near the bottom of the photographs.

We ran a cable (2-mm-thick wire rope) horizontally between the 2 trees and high enough that the bottom of the bait would be about 70–90 cm above the distal end of the run pole. Heavier pieces of bait required a higher horizontal cable. For bait, we used parts of deer, moose, black bear (*Ursus americanus*), or beaver (*Castor canadensis*) carcasses with a major bone attached. Baits were either road-killed animals or the remnants of animals taken by hunters or trappers. We threaded a piece of cable through a hole drilled through bone in the bait and, using a cable clamp, attached the bait cable to the horizontal cable and adjusted the height and location of the bait above the distal end of the run pole before tightening the cable clamp. We sometimes placed baits inside a cloth bag, under an inverted plastic bucket, or inside a metal paint bucket with lid if another species became habituated to the site and routinely removed the bait. We deployed and maintained the camera stations using boats, snowmachines, 4-wheelers, or helicopters for access.

We used the photographs obtained in 2008 to estimate wolverine density in the study area for that year and focused our 2009 camera-trapping efforts on documenting sex and lactation for wolverines visiting the camera stations as well as validating the photographic method of identifying wolverines. Because of the expense of helicopter access, in 2009 we eliminated some camera sites farthest from Petersburg that required helicopter access if no uniquely-marked wolverines were documented in 2008 at these sites. However, regardless of distance from Petersburg, we included at least 1 camera station where an individual was recorded in 2008 in the 2009 camera array, so that we

had at least some chance of photographing the same wolverines in 2009 if they were still using the same areas. We used multiple cameras (usually 2 but as many as 6, including Trailwatcher and Reconyx) at the 2009 camera stations so that we could determine if wolverines, on some occasions, were visiting the sites but not climbing the run poles and to increase our chances of acquiring identifiable photographs for all individuals. To increase our chances of documenting the sex and reproductive status of the wolverines being photographed, we modified the run poles in 2009 (described below) with structures that supported the animals as they stood at the end of the run pole, which resulted in a wide stance and photographs of the abdominal region. We set the Trailwatcher cameras to flash day and night to provide the highest detail possible of the animals on the run poles.

Hair-snagging

In 2009 we added hair-snag devices to the same run poles used to photograph the wolverines. To attach the hair-snag structure (described below) to the pole, we used 2 short pieces of 2-inch x 4-inch dimensional lumber, one that was 35.6 cm (14 inches) long and one that was 40.6 cm (16 inches) long, and attached the shorter piece with screws horizontally to the run pole so that it was perpendicular to the pole, flush with the distal end of the pole, and centered on the pole. The longer crosspiece was positioned similarly but was 22 cm from the distal end of the run pole (Fig.8).

We used square rods (~ 2.5 cm) of ultra high molecular weight (UHMW) polyethylene plastic (Plastic Supply Inc., Tacoma, WA) and steel alligator clips with 7.9-mm jaw opening (Mueller Electric Company, Akron, OH) to construct hair-snag structures, which we mounted at the distal end of the run poles (Fig. 9). The support structure consisted of 1) 2 vertical 76-cm-long pieces of UHMW plastic (uprights), each screwed at their base to opposite ends of the shorter crosspiece on the run pole; 2) 2 similar pieces of UHMW mounted diagonally for bracing the 2 uprights (braces), one end of each piece bolted to the top of the UHMW uprights and the other screwed into the end of the longer wooden crosspiece on the run pole; and 3) a 46-cm-long, 6.4-mm-diam (¼ inch) threaded steel rod that spanned the distance between the uprights ~2.5 cm from the top of the structure (crossbar). We used 2 additional pieces of UHMW ~30–40 cm long

(snag posts) on which we mounted 5–8 alligator clips (Model #BU-60PR2) (Fig 10). We inserted the base end of the clips into 6.4-mm-diam ($\frac{1}{4}$ inch) holes drilled into the snag posts and, tapping on the point of the jaw lightly with a hammer, firmly seated the base clips into the holes. We screwed the snag posts vertically into the proximal edge of the longer crosspiece, one on each side of the run pole separated by ~25 cm (Fig. 10). When the snagging device was set, each of the base clips held open a trigger clip (Model #BU-60). We attached the trigger clips to Henssger simplex snaps® (Henssger Hardware Corporation, Glens Falls, NY) with lanyards of Steelon® nylon-coated wire (BD45BL) (Berkley®, Spirit Lake, IA) and Berkley connector sleeves (B3) and secured the clips to the snag posts by snapping the spring clip onto small eye screws inserted into the snag posts (Fig. 11). The springs in the base clips were at least as strong as those in the trigger clips so that the base clips held the trigger clips open to the widest extent possible. When dislodged by an animal, the trigger clips would dangle at the end of the lanyard, preventing another animal from depositing hair in the clip (Fig. 11). We positioned the bait about 30-40 cm above the crossbar.

When we visited the C&H stations to collect hair, which we did each time we checked cameras to change batteries and memory cards at 1–4-week intervals, we placed the hair from each trigger clip into a separate small envelope and labeled the envelope with an accession identification number (AIN) that included the date, camera site, and clip position on the snag post (e.g., C10-041809-R7 indicated a sample of hair collected at camera site C10 on 18 Apr 2009 from the seventh clip from the top on the right snag post). We then placed these envelopes into a larger envelope labeled with a collection identification number (CIN) that included the camera site and collection date (e.g., C10-041809). Because we had mounted the alligator clips so they could be seen on the photographs, we could tell which clips were set and which ones were triggered both before and after a visit by a wolverine (Fig. 12), thereby allowing us to determine which individual wolverine was associated with each triggered clip. This integrated method of photographing and collecting hair from wolverines simultaneously made it possible to link genotypes with the unique ventral patterns of the wolverines photographed at the sites.

Carcass Collection

We purchased wolverine carcasses from trappers in SE AK during trapping seasons 2006/2007– 2008/2009. From these carcasses, we collected tissue samples for DNA analyses of wolverine population structure in SE AK compared to adjacent regions. Additional tissue samples were archived at ADFG (Juneau) for future isotope analyses of food habits of wolverines in SE AK (isotope analyses were not part of this study). In addition, the reproductive tracts of female wolverines in the carcass collection were examined for evidence of past reproduction, and baculum length was measured on males as an indicator of the percentage of yearling males in the harvested sample. The carcasses were then sent to the Dr. Joseph A. Cook (Department of Biology, University of New Mexico[UNM], Albuquerque) where DNA was extracted and the skeletons were cleaned and deposited in the Museum of Southwestern Biology at UNM.

DNA Analysis

We collected 3 types of samples for DNA analysis: muscle tissue from wolverine carcasses purchased from trappers, hair from live-trapped wolverines captured in Thomas Bay, and hair from snagging devices on the run poles in our study area. Tissue samples and hair collected from live-trapped wolverines were sent to J. Cook and graduate student J. Frances, who completed a comparative study of wolverine population genetics in North America in which they discussed the level of interchange between wolverine populations in SE AK and Canada. Methodology for the DNA analysis is described in Frances (2008). We report the findings of this study that pertain to SE AK in the results section below.

Hair from snag devices on the run poles provided DNA that was used to validate our method of determining sex and individual identity (ID) of wolverines from photographs. To validate the accuracy of ID and sex determined from photographs of the wild wolverines, we compared results from photographs with that from DNA using the following protocol. From photographs with a full ventral pattern, A. Magoun determined ID of the wolverines and assigned a name for each individual with a unique ventral pattern. The sex of the individuals live-trapped in either 2007 or 2008 was known but, for all other wolverines, we determined sex from photographs of the wolverines in a bipedal

stance. The sex of wolverines could be identified from photographs when the animals stood up on the run pole to reach the bait while facing the camera (Fig. 13). The method of identifying sex from photographs was suggested by C. Long (The Wolverine Foundation, Inc., Kuna, ID) based on his work with captive animals. We developed our field method for obtaining sex from photographs by working with >30 captive wolverines of known sex (D. Pedersen, unpublished data) and developing a file of photographs of adult (≥ 3 years old) male and female wolverines. At the time of our tests, no captive wolverine was <3 years old, so for younger-aged animals, we used a time series of photographs of the ventral surface of hand-reared wolverines provided by C. Long, D. Pedersen, and S. Kroschel to determine whether young males (9 months to 3 years) could be reliably separated from females. We could determine sex even for yearling males using detailed photographs, but males ≥ 2 years of age were more easily identified because of the larger size of testes and prepuce (Fig. 13).

By examining the photographs, A. Magoun determined which clips were triggered by a particular animal and assigned the AIN for each clip to that animal. She then provided a list of the wolverine names and their sex with the associated CIN and AIN data to C. Long (The Wolverine Foundation, Inc., Kuna, ID) prior to DNA analysis of the hairs. Without knowledge of ID and sex of wolverines identified from photographs, Dr. Michael K. Schwartz and Kristy Pilgrim (Wildlife Genetics Laboratory, Rocky Mountain Research Station, Missoula, MT) extracted DNA from the hairs and identified the genotypes and sex of the individuals in each hair collection having suitable DNA. They assigned a code number to each genotype and provided a list of the DNA ID codes, sex, and associated CIN and AIN data to Magoun and Long, who independently compared the data files and then compared their individual results. Using this protocol, we avoided observer bias in the validation process.

Density Estimation

The model we used for estimating wolverine density in our study area was developed by Dr. J. Andrew Royle (USGS Patuxent Wildlife Research Center, Laurel, MD) and is a spatial hierarchical model (Royle and Dorazio 2008) using capture-recapture data (Royle and Young 2008) from our 2008 camera array. The model can be conveniently analyzed

using methods that are standard for analyzing generalized linear mixed models (Bolker et al. 2009) using a Bayesian approach (McCarthy 2007) and data augmentation (Royle et al. 2007, 2009) in the free software program WinBUGS (Gilks 1994). The model is described in a paper submitted to the *Journal of Wildlife Management* in February 2010 and the submitted version is attached in Appendix A.

The key idea in this spatial capture-recapture model is that it formally relates the encounters of individual wolverines to where these individuals spend time over the camera-trapping period. In particular, if we imagine that an individual occupies a defined area for a given period of time (i.e., a seasonal home range or use area) and its activities are centered in that area for that period, then it is natural to expect that the probability of an individual encountering a trap is a function of the distance between that individual's activity center and the trap. The estimate of density is based on the number and distribution of individual activity centers (a latent variable that is the quantity of interest in the modeling exercise). Past experience at a camera station will affect the probability that an individual will be "captured" (i.e., photographed sufficiently well to be identified) at that site again. Therefore, we incorporated a "behavior response" in the model that takes into account the fact that wolverines remember locations where they have found food in the past and return to these locations periodically, even long after the food is gone (A. Magoun, personal observation). Because the behavioral response is trap-specific, the probability of subsequent encounters is increased only for that trap. In this respect, the behavioral response can be considered a "local behavioral response" rather than a "global behavioral response" (i.e., encounter with one trap increases probability of being captured at other traps). Other factors that probably affected the probability of "capturing" a wolverine at a camera station, such as individual differences in personality, territoriality, sex, hunger, and reproductive condition, were largely unknown for the individuals in our study and therefore were not included in the model. Moreover, the interaction of these variables on detectability of individual wolverines is likely to be complex and to change over time.

Independently of the spatial hierarchical model, we used knowledge of wolverine spacing behavior (Persson et al. 2009; M. Aronsson, Grimsö Wildlife Research Station, Sweden, unpublished data) and information from radiotelemetry and cameras over the 3

years of the study to develop an ad hoc model of wolverine spatial structure and density in the study area for 2008. We considered wolverines that were known to occupy the study area in 2 consecutive years in the period 2007-2009 to be residents in 2008. Based on home range size of collared animals and known locations of all wolverines detected in 2008, we constructed a map of distribution and hypothetical home range boundaries for the resident animals. We calculated a minimum wolverine density for our study area by dividing the size of the study area by the number of residents detected.

RESULTS

Livetrapping

In the 2006-2007 field season (hereafter referred to as 2007), we deployed live traps at 8 sites and operated them for a total of 246 trap nights from 30 December 2006–2 April 2007. Wolverines triggered the traps 7 times, resulting in the capture of 3 males and 1 female (Table 1). One of the males was recaptured in the same trap a week after its initial capture. This wolverine had numerous fresh wounds and old injuries (Fig. 14) that prevented us from putting a telemetry collar on the animal. We obtained no more locations for this individual either in traps or in photographs. Wolverines escaped twice by chewing out of the live traps. Photographs from cameras set up at the trap sites indicated that at least 2 individuals visited the trap sites but were not captured, either because the trap was not set at the time or the animal failed to trigger the trap. Video footage showed one individual entering and leaving the trap without triggering the trap.

In the 2007-2008 field season (hereafter referred to as 2008), we deployed live traps at 12 sites (including the 8 from the previous season) and operated them for a total of 301 trap nights from 18 January 2008–20 April 2008. The least number of trap nights for a given trap site was 3 trap nights with 1 wolverine captured; the longest number of trap nights was 86 with 2 captures (both recaptures from other trap sites). Wolverines were captured on 8 separate occasions, of which 3 were recaptures of previously collared animals. Of the 5 wolverines collared, 2 were adult males (i.e., ≥ 2 years based on large testes size), 2 were reproductive females (i.e., teat development indicating past reproduction), and 1 was a nulliparous female (Table 1). The female *Sunshine* was

lactating at the time of her capture on March 28 (Fig. 15). The male *Moonshine* had been captured and collared the previous year but was no longer wearing the collar.

Argos/VHF and VHF-only Collars

In the first live-trapping season, 2 wolverines (male *Dave* and female *Cean*) removed their collars within days of their captures so we obtained very few locations for these animals in 2007, but we subsequently photographed *Cean* in 2008 and 2009. We did not detect the male again. We placed the third collar on the adult male *Moonshine* captured on 23 February 2007 and that unit remained on and functional for over a month. From April 3–April 30, we received 83 locations from the Argos transmitter on this male, with an average of 3 locations per day. There were no locations on 3 days during that period. The highest number of locations for any single day was 10. Most of the Argos locations (82%) were in the A or B location classes (i.e., accuracy unknown). Because of the unknown error in Argos locations in categories A and B, we based analysis of home range size on categories 1, 2, and 3 with estimated errors: category 1 <1000 m, category 2 <350 m, and category 3 of <150 m. To obtain a sample of accurate locations, we radio-tracked the wolverine from the air 15 times during this 28-day period using the VHF signal.

In 2008 we fitted 4 animals with Argos/VHF collars, but one of these collars failed after only a couple of weeks so the male *Beaverdam* was recaptured and collared with a VHF-only collar. The nulliparous female *Snowstorm* was the last animal captured and was also outfitted with a VHF-only collar. Of the remaining 3 wolverines fitted with Argos/VHF collars in 2008, one was the adult male *Moonshine* also collared in 2007 and 2 new animals, a lactating female *Sunshine* and another adult female *Mariner* whose teat development indicated she had reproduced in the past. *Sunshine* apparently shed her collar or died sometime in late May; the VHF signal indicated the collar was no longer moving (mortality mode). She was not subsequently photographed even though cameras she had visited previously were still deployed in her home range after the mortality signal was detected. We were not able to retrieve the collar. Before the collar stopped moving, we were able to locate the natal and maternal den sites she was using from her capture date on 28 March 2008 until her last known location. Female *Snowstorm* also

shed her VHF collar sometime in late May and an attempt to retrieve the collar was unsuccessful; this female was later photographed at a camera site without the collar. Satellite reception of signals from the Argos transmitters varied among collars on different wolverines. Very few signals were obtained from the lactating female *Sunshine*, probably due to steep terrain around her den site or poor signal reception from under snow, trees, and rocks where she denned. Satellite reception from the other adult female *Mariner* was good and indicated that she was probably not denning, a conclusion supported by VHF relocation data. Satellite reception on the adult male *Moonshine* was relatively poor in 2008 compared to a similar collar he wore in 2007. Reasons for the poor reception were unknown, but VHF locations showed that he spent more time in mountainous habitat in 2008 than in 2007. VHF signal reception was good throughout the tracking period and on only a few occasions were we unable to locate collared animals, usually because inclement weather prevented us from searching widely for the signal.

Home Range and Movements

Figures 16–18 and 20–23 represent the core areas used by collared wolverines in 2008 and in 2007 for *Moonshine*, an adult male that was the only wolverine tracked during both years of study (Fig. 16). *Moonshine*'s home range shifted in 2008, indicating an avoidance of the northwestern end of his 2007 home range (Fig. 16). There was no overlap of *Moonshine*'s 2008 home range with that of the 2008 home range of the adult female *Mariner* to the west (Fig. 17). *Moonshine*'s 2007 and 2008 home ranges included a camera site where adult female *Zigzag* was identified from photographs in all 3 years of the study. In 2009 when *Zigzag* was identified from photographs as a lactating female, *Moonshine* was photographed at 2 camera sites visited by this female, indicating that the home ranges of these 2 wolverines overlapped at least in 2009 and probably earlier as well. *Moonshine* had a home range of 527 km² in 2008 and none of this MCP included large bodies of water.

In contrast to *Moonshine*, the adult male *Beaverdam* had a home range of only 163 km², which would have been even smaller if we had subtracted open water from the calculation of home range size (Fig. 18). This male did not display the typical movements and home range characteristics of a resident adult male. He spent weeks in very small

areas, sometimes swimming short distances (<400 m) to near-shore islands and made occasional, relatively rapid movements around the perimeter of Thomas Bay. He was documented only once in the mountainous portion of the study area, where he was photographed at one of the camera sites. This animal had large, recent wounds on his back and face when recaptured on 3 April 2008 (Fig. 19).

Home ranges for female wolverines were larger than the home range for *Beaverdam*. The adult female *Mariner* had a home range of 308 km² with no large bodies of water within her MCP (Fig. 20). Interestingly, this parous female was initially captured in Thomas Bay on 27 February 2008. Upon release she moved westward over 20 km to her core home range in a 24-hour period (Fig. 21) and was not documented outside of that home range again from March–July 2008 or on cameras outside that range in 2009. Photographs from the motion-detection cameras indicated the presence of at least 4 other wolverines within *Mariner*'s home range in 2008 (*Sarge*, *Hoot*, *Morse*, and *Splish*), but we only determined the sex of 2 of these individuals, one of which was a non-lactating female (*Sarge*) and the other a male (*Hoot*). The non-lactating female *Snowstorm* had the largest home range at 631 km², but the MCP included portions of the ocean (Fig. 22). We did not detect *Snowstorm* in the study area in 2009. Her home range overlapped those of the other 4 radiocollared wolverines. As mentioned above, we did not have enough locations to determine home range size or extent for the lactating female *Sunshine* in 2008 (Fig. 23).

Motion-detection Cameras

In addition to cameras deployed for monitoring activity at live traps in 2008, we established 37 camera stations with Trailwatcher cameras in a camera array with a convex hull of 1,549 km² and a camera density of 24/1,000 km² (Fig. 24). The closest 2 (i.e., nearest-neighbor) cameras (#1 and #8) were 0.6 km apart (separated by a 250-m-wide channel between Ruth Island and the mainland). The greatest nearest-neighbor distance was 9.6 km (#33 and #34). The cameras were operational for 5 to 108 days (median = 45 days) over a 165-day period (19 January to 13 June). We used the data from this camera array to calculate density of wolverines in the study area in 2008 (see density estimation below).

We obtained over 3,000 photographs in 2008 that showed at least a portion of a wolverine from the cameras. Wolverines were photographed at 27 of the 37 (73%) camera stations and 21 individuals were identified from photographs (Fig. 25), including all 5 of the animals collared in 2008 (mean = 0.57 individuals/camera). Two individuals live-trapped in 2007 (*Lucky* and *Dave*) did not show up at any camera site in 2008.

The number of photographs taken of an individual wolverine during a “visit” to a camera site ranged from 1 to 218 with an average of 20 photographs per visit. We defined a visit as the period between the first photograph and the last photograph taken of an individual wolverine before there was more than a 30-min break between photographs. Some wolverines spent less than a minute at the camera site while one spent as long as 218 minutes. The highest number of visits by a single individual in a single day was 4. The same wolverine sometimes visited the same site on as many as 4 consecutive days. The highest number of individuals documented at any one site was 4. It was not uncommon for 2 different wolverines to be photographed at the same site on the same day. Sometimes visits by 2 different wolverines were made within an hour of each other. In one case, 2 wolverines were photographed at a camera during the same visit, although they were not photographed together on the run pole. On 6 occasions, an individual was detected at 2 different camera sites on the same day. The greatest straight-line distance between these camera sites was 11.7 km and the time between the visits to these 2 sites was 17 hr. The briefest time between visits by the same individual to 2 different cameras on the same day was 1.5 hr for 2 cameras that were 3.4 km apart. On 2 occasions, wolverines were photographed at sites within an hour after our visit to maintain the camera. We were able to identify 2 lactating females (*Cean* and *Sunshine*) using the cameras during March-May 2008.

In 2009 we eliminated 13 stations from the 2008 camera array and added 5 new camera stations (accessible by boat and 4-wheeler) for a total of 29 sites in the 2009 camera array (Fig. 26). We used multiple cameras (Trailwatcher and Reconyx) at the camera sites so that we could collect information on sex and reproductive condition and determine if wolverines were visiting the sites but not climbing onto the run poles. Wolverines were photographed at 22 of the 29 (76%) sites and we identified 18 individuals (average = 0.62 individuals/camera) (Fig 27).

Besides wolverines, martens (*Martes americana*), black bears, and bald eagles (*Haliaeetus leucocephalus*) accounted for most photographs on the cameras at the C&H stations. Among the species that also triggered the cameras while on the run poles were mice (*Peromyscus maniculatus*), red squirrel (*Tamiasciurus hudsonicus*), flying squirrel (*Glaucomys sabrinus*), weasel (*Mustela erminea*), mink (*Mustela vison*), porcupine (*Erethizon dorsatum*), crow (*Corvus caurinus*), Steller's jay (*Cyanocitta stelleri*), and smaller bird species. Moose and deer sometimes triggered the cameras as they walked by. Only a single wolf (*Canis lupus*) was photographed at a C&H station in June 2009 as it walked by a camera.

Hair-snagging

In 2009 we collected 188 hair samples in 41 collections at 18 of the 29 (62%) C&H stations. After examining the photographs taken at the C&H stations, we selected 35 collections for which we could determine which animal triggered the clips on the hair snagging device. In all but one collection, only 1 wolverine was responsible for triggering the clips with hair. In collection C36_042509, 2 wolverines, *Beaverdam* and *Sarge*, triggered clips (Table 2).

DNA Analysis

Population Structure

We took tissue samples from 23 wolverine carcasses purchased from trappers across SE AK in 2007 and 2008. The DNA analysis from the carcass collection, as well as from carcasses from adjacent Canadian provinces of Yukon and British Columbia, provided the basis of a Masters thesis by J. Frances (University of New Mexico, Albuquerque) (Appendix B). The subject of the thesis was an analysis of the spatial structure and demographic history of wolverine in North America with an emphasis on peripheral populations, including SE AK. Results of this research that are particularly relevant to the objectives of our study of reproductive females in SE AK are the following:

- 1) corroboration of previous studies indicating female genetic variation is spatially partitioned in wolverines (Tomasik and Cook 2005, Cegelski et al. 2006, Schwartz et al. 2007),

- 2) barriers to gene flow seems to have a stronger effect on spatial genetic variation than geographic distance,
- 3) in contrast to another relatively isolated population on the Kenai Peninsula, Alaska, the wolverine in SE AK has 2 private alleles, indicating possible reduced gene flow with neighboring Canadian populations and a partially isolated population,
- 4) corroboration of Tomasik and Cook (2005) in which they recognized the SE AK wolverine as a distinctive population,
- 5) island and mainland populations in SE AK are not distinctive, which is to be expected because wolverines only occur on nearshore islands within the Alexander Archipelago,
- 6) SE AK wolverines do not appear to be a post-glacial refugial population, and
- 7) migration to and from SE AK appears to be male-biased, which could prevent inbreeding depression, although lower numbers of immigrant females may have implications from an ecological standpoint when populations are intensely harvested.

DNA Verification of Identity and Sex of Photographed Wolverines

To reduce the cost of DNA analysis, we selected 35 collections for which our records indicated we had identifiable photos of wolverines associated with them and we could determine which triggered clips were associated with the photographed animals. We subsampled in each collection when photos showed that only 1 individual had visited the site before the hair was collected or, in the case of a visit by >1 individual, we could see in the photos which wolverine had triggered which clips. If we could not match a wolverine with a particular clip and >1 individual had potentially contributed hair to a single collection, then all samples in the collection were analyzed, but we had only 1 occurrence of >1 individual's DNA represented in a single collection (*Beaverdam_M8* and *Sarge_F7*; Table 2). The Wildlife Genetics Laboratory performed DNA extractions on 74 samples using 9 variable microsatellite loci and obtained quality DNA for genotyping 37 of the 74 samples (50%), which represented 28 of the 35 collections we had selected (80%).

We obtained identifiable photographs of wolverines at 22 of 29 (76%) stations. From photographs, we identified 14 individual wolverines that were associated with hair samples collected at the C&H stations (8 M, 6 F), while from DNA analysis of the hairs, we identified 13 individuals (8 M, 5 F). The DNA quality was too poor for DNA identification of female *Zigzag*. For those collections with good quality DNA, there was 100% agreement between the photographic data file and the DNA data file, both in sex and individual identity. Six other collections also had poor quality DNA but we identified the individuals associated with these collections using photographs and so were able to assign a genotype to these collections. Two of the animals in the photographs wore radiocollars (*Moonshine* and *Mariner*). Therefore, we based our test of sex identification from photographs on the remaining 11 individuals that we genotyped. Our determination of sex from photographs matched that from DNA in every instance.

Of the 13 individuals identified by both photographs and DNA, we identified 5 in only 1 collection and 8 in 2–6 collections (Table 2). All individuals identified from multiple collections were identified by both photographs and DNA in the same collections. In addition to the 14 individuals photographed at stations where hair was collected, 4 other individuals identified from photographs at C&H stations (1 M, 1 F, and 2 of unidentified sex) could not be genotyped because either the triggered clips had not snagged hair, the clips were already triggered by another animal, or the wolverines failed to trigger clips. We submitted a paper to the *Journal of Wildlife Management* on 2 February 2010 on this method of integrating cameras and hair snags for detecting wolverines (Appendix C).

Density Estimation

Using the photographs from the 2008 camera array and a spatial capture-recapture (SCR) model (Appendix A), we estimated the density of wolverines in the study area at 9.7 wolverines/1,000 km² with a Bayesian confidence interval (BCI) of 5.9–15.0. Although we identified 21 wolverines from photographs in 2008, not all of these individuals were known unequivocally to be residents in the study area throughout the camera-trapping sessions. We determined that a minimum of 18 of the 21 wolverines were resident in the study area in 2008 using the ad hoc model derived from telemetry, photographs, and

knowledge of wolverine spacing patterns over the period 2007–2009 (Fig. 28). We excluded 3 wolverines that had been photographed in 2008 (*Chuck*, *Splish*, and *Splash*) for which there was no evidence of their presence in the study area for 2 consecutive years. If these 3 additional wolverines had been residents entirely within the study area in 2008, the ad hoc estimate of density would have been 9.8 wolverines/1,000 km², very close to the estimate using the spatial hierarchical model. By excluding these 3 individuals and assuming no wolverines went undetected by our cameras, we derived a minimum density estimate of 8.4 wolverines/1,000 km² for the 2,140 km² study area, considerably higher than the low estimate of 5.9 wolverines/1,000 km² for the SCR model.

Carcass Collection

Besides the 23 carcasses used in the genetic analysis mentioned above (Frances 2008), we collected an additional 13 wolverine carcasses from trappers in Southeast Alaska from 2006/2007–2009/2010 trapping seasons. Most of the purchased carcasses were males, and very few of the carcasses from females showed indication of past reproduction. Analysis of these carcasses is ongoing.

DISCUSSION

Livetrapping

Evidence from our cameras indicated that some wolverines remained very timid around the traps while others readily entered the traps even after they had been caught and radiocollared. Unless information from VHF or satellite collars is needed for specific research goals, such as location of natal dens, the C&H stations designed for this study provide a more effective and efficient method for obtaining population parameters for wolverines in remote areas that are difficult to access, especially for long-term monitoring studies. In two winters, we obtained information on only 8 wolverines using live-capture techniques but were able to obtain information on 25 wolverines with C&H stations.

Argos/VHF and VHF-only collars

The Argos/VHF collars were adequate for obtaining general information on the location and movements of wolverines, except for the denning female *Sunshine*. Argos locations for this female were concentrated at her den sites during the denning period but because the female spent so much time at the den site during the tracking period, little information was available on the size and boundaries of her home range. VHF locations were also concentrated at the den site during the period the collar was transmitting on active mode. Unfortunately, no more locations were possible for this female after the collar began to transmit in mortality mode in May.

Although VHF locations were more expensive to acquire per location, detailed information on habitat use and movements was possible using aerial radiotracking, and we were able to track the wolverines over a longer period of time because the Argos transmitters only operated for approximately 4 months compared to 1 year for the VHF transmitters. None of the collared individuals dispersed out of the study area during the period the radiocollars were transmitting so it was not necessary to use Argos transmitters to follow dispersing individuals, which is one of the main advantages of these satellite transmitters. If the main goal of a telemetry study is to locate natal dens and obtain information on resident female wolverines, we suggest that VHF telemetry is more useful than Argos satellite collars. GPS-collars downloadable to an Argos satellite show promise as the ideal telemetry platform for wolverines but these units are still too large and bulky for wolverines.

Home Range and Movements

Centers of activity based on Argos locations showed no substantial deviations from 100% MCP home ranges derived from VHF relocations, indicating that the areas depicted in Figures 16–18 and 20–23 are accurate representations of the center of activity of the collared animals during the period their transmitters were operating. Home range size for 4 individuals with at least 20 relocations from March through July falls within the published home range sizes of wolverines in other study areas (Persson et al. 2009), except for the adult male *Beaverdam*. This male did not display the typical movements and home range characteristics of a resident adult male. The recent wounds on his back

and face when recaptured on 3 April 2008 indicated that he probably had been fighting with another wolverine. All these factors suggest that this male was likely a “floater” (i.e., non-territorial individual) moving through territories of other wolverines. The nulliparous female *Snowstorm*, having the largest home range of the radiocollared wolverines, was likely a “floater” as well and moving through the territories of other females (M. Aronsson, Grimsö Wildlife Research Station, Sweden, unpublished data).

Adult male wolverines defend territories that contain the territories of a number of adult territorial females (Persson et al. 2009) and wounding on wolverines captured and photographed in our study area indicated that intraspecific aggression increases in late winter. Of the 5 radiocollared wolverines that were tracked during late winter in our study area, only one, *Moonshine*, appeared to be a territorial adult male. In 2007 *Moonshine*’s territory included the area used by *Mariner* in 2008 and probably used by her in 2007 as well. We believe that *Sarge*, detected on camera in 2008, was *Mariner*’s daughter and would have been born in 2007. *Moonshine* did not include *Mariner*’s home range in his own home range in 2008, however. Instead, in 2008, *Moonshine* concentrated his movements in a portion of the study area (Fig. 29) that included an area used by *Zigzag* (a parous female) and an undetermined female to the northeast, possibly *Skinny*. We photographed *Skinny* over a 2-year period in this area and detected a new female (*Glacier*) at the same camera site in 2009, which could have been a daughter of *Skinny*. Unfortunately, we were not able to verify lactation or collect hair from *Skinny* for DNA analysis. If *Zigzag*, *Mariner*, and *Skinny* were territorial females on the north side of Thomas Bay in 2008, then it is probable that another, undocumented territorial female occupied the area east of *Mariner* in 2008 (Fig. 30). It is possible that *Morse* was the territorial male in this area in 2008, an area not used by *Moonshine* in either 2007 or 2008, but we were not able to verify sex of *Morse*. He was detected in this area in 2009 as well.

Density of cameras and number of operational days was greater on the south side of Thomas Bay than on the north side. We believe we probably documented all territorial females in this area. Furthermore, because *Paleface* was documented in all 3 years of the study in the northern portion of this area, we believe he was the territorial male in the home range of *Sunshine* and *Cean*. Another male, *Little Spot*, was photographed in the

southern half in all 3 years of the study. DNA analysis of the genetic relationship of the wolverines in our study area is ongoing.

Hair-snagging

Using photographs that showed the individual that triggered the alligator clips, we were able to subsample from between and within the hair collections without compromising our ability to identify all individuals that left hair at the C&H stations. This is one advantage of using alligator clips rather than barbed wire for snagging hair from wolverines (e.g., Mulders et al. 2007, Fisher et al. 2009). Moreover, we believe that alligator clips are superior to barbed wire in ease of deployment and the additional information provided by linking DNA identity with photographs of the individuals. By associating a genotype with a unique ventral pattern, subsequent hair collections can be sorted by individual and would not require DNA analysis if photographs indicate a known individual. Effort can then be concentrated on obtaining hair at C&H stations where photographs indicate wolverines without a genotype are using the site, including new individuals that may be offspring of known individuals. For large-scale or multi-year studies, limiting the number of hair samples that must be analyzed for DNA while ensuring a comprehensive database of individuals in the study could potentially save thousands of dollars in laboratory costs.

In this study, we linked genotype to 5 individuals identified in photographs by their ventral patterns using a single hair collection each. However, we recommend that, whenever possible, at least 2 hair collections are used to link ventral patterns to genotype to cross-validate the linkage. We also strongly recommend that only the full frontal ventral patterns (i.e., all of the throat and chest) be linked to genotype to avoid incorrect associations in subsequent collections of photos and/or hair (see discussion on photographing wolverines at C&H stations). Furthermore, if there is any doubt that a particular alligator clip was triggered by the wolverine in the photograph, then at least one more additional hair collection for that individual should be analyzed for genotype. Loose hairs on the hair-snag structure should not be used to link genotype with a particular wolverine, even if no other wolverine was photographed at the site, to prevent mistakes in matching DNA to the correct wolverine. Even if photographs indicate that

loose hairs are almost certainly from a particular individual, verification from another collection should still be made before assigning a final genotype to that individual.

Motion-detection Cameras

Motion-detection cameras made it possible to identify 21 different wolverines at C&H stations in 2008 and an additional 4 individuals in 2009. Because live-trapping wolverines was not feasible in the remote mountainous portions of the study area, documenting wolverines with motion-detection cameras and/or DNA from hair samples was the only way to determine the distribution and number of wolverines using the area. The initial expense of cameras was off-set in this multi-year study as we gained more information from the cameras each year and the photographs made it possible to select among hair samples for DNA analysis, which considerably reduced our laboratory costs.

The features we found to be most important in selecting a camera model for documenting ventral patterns, sex, and lactation in wolverines were 1) ability to focus quickly and consistently provide sharp photographs, 2) flash selection day and night, 3) dependability in inclement weather, 4) large capacity memory cards that outlast the charge on the batteries, and 5) good service from the supplier. Ease of operation and small size of the units were also advantages. However, there was no perfect camera on the market. Cameras that use mechanical parts such as lens covers that open and close or lenses that focus by moving in and out (e.g., Trailwatcher) must be reset after spells of cold weather in which temperatures fall below -23° C. Some wolverines may be intimidated by cameras with a white flash or by the sound of the cameras operating, and wolverines may need to become accustomed to them before they will spend time on run poles. While the infrared Reconyx cameras are not affected by cold weather and do not flash (except for the infrared glow), they are more likely to produce blurry photos, photos in black and white with less detail, and have no white flash to fill in shadows or produce sharp color photos under all lighting conditions. In our experience, most wolverines became accustomed to the white flash of the Trailwatcher cameras, but we recommend that after a wolverine makes 2 visits to a C&H stations with a Trailwatcher (or other white flash camera) without attempting to access the bait, the Trailwatcher should be replaced with a Reconyx camera until the ventral pattern is photographed and the

wolverine accesses the bait. Afterwards, the Reconyx can be replaced with a Trailwatcher for more detailed photographs.

DNA Analysis

Population Structure

Preliminary results of DNA analyses from wolverine carcass collections suggest that the glaciated portions of the SE AK panhandle may serve as a partial barrier to dispersal for the female segment of the wolverine population, thereby limiting interchange between SE AK and adjacent Canadian province (Frances 2008). Although there is no reason to assume that females are less able than males to traverse the glaciated portions of SE AK, a number of factors may make it less likely for females than males to immigrate into SE AK from Canada. Female wolverines often inherit their mother's home range or establish a home range adjacent to their mother (M. Aronsson, Grimsö Wildlife Research Station, Sweden, unpublished data) while male offspring always disperse to new areas. Moreover, because territorial males have home ranges that are several times larger than those of territorial females, there are more non-resident males in the population. Therefore, immigrants from Canadian provinces are more likely to be males. Further studies are needed to clarify the difference, if any, in dispersal patterns of male and female wolverines in SE AK and adjacent provinces. Analysis of population viability and sustainable wolverine harvests must take into consideration the fact that movements of wolverines into and out of SE AK may be low relative to many other wolverine populations in Alaska and Canada.

DNA Verification of Identity and Sex of Photographed Wolverines

The integrated system of cameras and hair snags that we tested during this study provides a new, non-invasive method for obtaining information on wolverine populations that will be useful for conservation and management of this species. Besides providing information on ID, sex, and genotype as in this study, the C&H monitoring technique has the potential to provide data on familial relationships and movement patterns of uncollared individuals based on DNA, survival rates in multi-year studies, lactation in females, reproductive status of males, centers of activity, population density using

capture-recapture models, and behavioral information such as association of individuals based on the date and time stamp on photographs. In fact, we believe that the full value of the system will not be realized until other researchers and managers apply the technique to their particular conservation and management needs. Additional information on familial relationships and survival of wolverines photographed at the C&H stations will be forthcoming from this study.

Density Estimate

The estimates of wolverine density for our study area in 2008 using both the spatial hierarchical model and the ad hoc model combined provided a density estimate that is at the upper end in the range of densities published for this species. The lower BCI for the spatial hierarchical model (5.9/1,000 km²) is well below the known minimum density of 8.4 wolverines/1,000 km² derived with the ad hoc model and based on identified resident wolverines in 2008. Therefore, the actual density for wolverines in the study area in 2008 probably falls between 8.4 and 15.0 wolverines per 1,000 km². The upper BCI (15.0/1,000 km²) is higher than the highest densities reported from other studies (Persson 2005, Golden et al. 2007, Mulders et al. 2007). Even if we had counted all wolverines photographed in 2008 as resident animals, our ad hoc estimate would have been 9.8/1,000 km². A density of 15.0 wolverines/1,000 km² would have required an additional 11 undetected, resident wolverines $\geq$ 1-year-old in the study area during the camera-trapping period, and this is very unlikely given the spatial pattern of the animals that were detected [Note: we did not include newborn wolverines in either of the density estimates because these kits would not be detectable on cameras during the camera-trapping period, and we did not attempt to document kits at dens].

We probably did not photograph all residents in the northern portion of our study area because we could not check these camera stations as often as we checked camera stations in the southern portion, which reduced the number of functional days for the northern cameras. Of the 14 camera stations north of Farragut Bay, 10 (71%) functioned for <46 days, while south of Farragut Bay, only 9 of 23 cameras (39%) functioned for <46 days, which was the average number of functional days for all cameras. As mentioned above, the radiocollared adult male *Moonshine* made regular trips into the area

north of Farragut Bay, an indication that at least one parous female (possibly *Skinny*) probably occupied this area and possibly offspring of this female. In addition, there may have been another adult female and offspring in the area immediately east of *Mariner's* home range (Fig. 30). However, resident females may be temporarily absent from previously occupied territories and not immediately replaced by another female (Aronsson 2009), as it appears to be the case for *Sunshine*, a radiocollared lactating female that probably died in May 2008 and was not replaced in 2009 (see discussion in the next section). Based on all the evidence, we suggest that an estimate of 9 wolverines/1,000 km² is a reasonable conservative estimate of density for our study area, at least under conditions that prevailed in 2008.

We expect that the density of wolverines in our study area fluctuates over time depending on the survival and reproductive rate of resident animals and the rate of immigration and emigration. Therefore, the density estimate for 2008 should be considered only a point estimate and was probably influenced by the relatively low harvest of wolverines in the study area in 2007 and 2008. However, the density of parous females is more important than overall density of wolverines in the study area because survival of adult female wolverines is the most important population parameter in viable populations of wolverines (Magoun 1985, Persson 2003). Of the 21 wolverines that were photographed in the study area in 2008, 4 (*Mariner*, *Zigzag*, *Sunshine*, and *Cean*) were known to be parous females and we suspect that at least one other, either *River* or *Cowgirl*, was also a parous female (the other her daughter). Finally, it is possible that *Skinny* was also a parous female but photographs were inconclusive. Based on information from radiotelemetry, camera trapping, information on wolverine spatial structure, and habitat features of the study area, the density of territorial females in the study area was probably between 2–4/1000 km² and that density is likely to remain fairly stable as long as food resources are stable and the harvest rate of parous females remains low.

Habitat Associations and Movement Patterns of Reproductive Female Wolverines

In summary, based on our radiotelemetry data, photographs from C&H stations, and knowledge of wolverine spatial structure (M. Aronsson, Grimsö Wildlife Research

Station, Sweden, unpublished data), at least 7 reproductive females probably lived entirely within the study area in 2008, and 6 of these 7 females were also detected in 2009. We believe that *Sunshine* died during May 2008 because her transmitter was heard on mortality mode and she was not photographed again either in 2008 or 2009. Cameras in her old home range did not detect another female there in 2009. Because habitat at the northern end of the study area is continuous with habitat north of the study area, the wolverine *Chuck*, who was photographed at a single C&H station at the northern tip of the study area in 2008, could have resided largely outside the study area and was not photographed in 2009 at that station. In 2008 there were 3 additional females in the study area: *Sarge*, residing within the home range of *Mariner*; *Snowstorm*, a radiocollared female that ranged across the home ranges of several females; and *Cowgirl*, photographed at a site where *River* was also photographed. DNA analysis of the familial relationships of these females is ongoing. The female *Snowstorm*, who appeared to be nulliparous, was not photographed again in 2009 and possibly dispersed outside the study area or died. In 2009, a new female was photographed in the home range of *Skinny*. If this female, *Glacier*, was a daughter of *Skinny*, then by the end of our field work in June 2009, there were at least 3 females present within the study area that could potentially replace *Sunshine*, or any of the other reproductive females, without the need for dispersers from outside the study area. In saturated wolverine populations, deceased territorial females are usually replaced by daughters or by dispersers rather than the empty territories being absorbed by a neighboring female; in Sweden 53% of the replacers were daughters (M. Aronsson, Grimsö Wildlife Research Station, Sweden, unpublished data). The Swedish study showed, however, that only 31% of the females reoccupying vacated territories produced young in their first denning season. The mean time before there was another reproduction within the territory of a replaced female in Sweden was 2.2 years (range 0–7 years). There is no information on what replacement time is for harvested populations or for populations that are geographically isolated.

We determined the location of one natal den during the study, in a stand of trees upstream of Scenery Lake at an elevation of ~350 m. Snow was very deep at this elevation and lasted well into June. Other reproductive females probably also denned at higher elevations, particularly in steep-sided mountain valleys where snow is still deep in

May. Photographs of the reproductive females were more frequently obtained at camera sites at elevations >300 m. For example, the female *Cean* was lactating in 2008 and 2009 and was photographed most frequently upstream of Swan Lake in both years, where we suspect she had a den in those years. Den sites are probably not limiting for reproductive females because of the abundance of snow in early spring, avalanche debris, large fallen trees, and rocky areas known to be used as den sites by wolverines (Magoun and Copeland 1998).

We believe the distribution of reproductive females is largely influenced by the distribution of mountain goats in the study area and that mountain goat numbers need not be high to support a reproductive female. For example, the Fanshaw Peninsula south of Port Houghton has some of the lowest mountain goat numbers in the study area (ADFG unpublished goat survey data), yet reproductive female *Mariner* and another female (*Sarge*) maintained home ranges on the Fanshaw Peninsula (Fig. 30) in both 2008 and 2009. We believe *Sarge* is a daughter of *Mariner*, probably born in late winter 2006, indicating that *Mariner* reproduced successfully in this area despite relatively low goat numbers.

There are no population estimates for other ungulates in the study area. Deer and moose probably occur in the diet of wolverines in some portions of the study area, but they are not likely to be as important as mountain goat in the diet of reproductive females in our study area. Other potential food items include ptarmigan (*Lagopus* sp.), blue grouse (*Dendragapus obscurus*), porcupine, beaver, marmots (*Marmota caligata*), and other small mammals. Marmots may be especially important for reproductive females raising young during the summer (Lofroth et al. 2007). Wolverines may also scavenge carcasses of black bears, which are common in the study area, and sometimes die in winter dens or are killed by other black bears. Bear hunters leave portions of bear carcasses in the field, mostly along beach areas during the spring bear-hunting season, but usually these carcasses are rapidly consumed by bald eagles (D. Benitz, personal communication). Streams in the study area support salmon runs in the fall and occasionally sea lion carcasses wash up along the beaches in the study area (D. Benitz, personal communication), so we assume that marine sources of food are seasonally or periodically available for some wolverines in the study area, although they are unlikely to

be critical food items in the diet of reproductive females during most of the denning and kit-rearing period.

Because suitable habitat for wolverines in our study area occurs in a relatively narrow band between the coast and the Stikine Icefield, home range boundaries of most reproductive females in the study area include a portion of the coast that is accessible to trappers by boat (Fig. 30). We detected all of the reproductive females except *Skinny* at C&H stations at sea level during the study. Even when a home range does not include part of the coastline, females may occasionally travel outside their home range boundaries. We captured the female *Mariner* in a live trap in Thomas Bay in February 2008 even though her home range (based on radiotelemetry and photographs) for the rest of 2008 and for 2009 was on the Fanshaw Peninsula. She would have had to travel through or along the boundary of the home range of *Zigzag* to access our live-trap site in 2008. From our analysis of probable home range boundaries and movements of reproductive females, we concluded that all wolverines in the study area could potentially be exposed to trapping if trappers were active along all areas of the coast from LeConte Bay to the head of Port Houghton. However, wolverine harvests from the study area have been limited to Thomas Bay and LeConte Bay in recent years.

CONCLUSIONS

Habitat associations, spatial structure, and ecological requirements of reproductive female wolverines in our study area, and probably on the mainland of SE AK in general, appear similar to that of other montane populations of wolverines in North America (Brock et al. 2007, Copeland et al. 2007, Krebs et al. 2007, Lofroth and Krebs 2007), with mountain goats being the main ungulate associated with the distribution of reproductive females across the study area. Snow cover in early spring was more than adequate for denning females, as were snow-covered denning structures such as downed trees and rocky areas.

Wolverine density in the study area in 2008 was among the highest reported for wolverine populations, and the density of reproductive females was probably near the maximum the study area could support, given the even distribution of females in the study area, the low mortality of females during the study, and low trapping pressure

before and during the study. Trapping pressure, particularly on reproductive females, appears low across most of SE AK at the present time. In 2009, a reduction in the length of the trapping season for wolverines in SE AK provides further protection for reproductive females during the denning season. Future analyses of wolverine harvests should include a method for determining the number and percentage of parous females in the harvest, and management objectives should focus on limiting the take of parous females if harvest levels increase appreciably in SE AK.

Possible resource development on the mainland of SE AK, including logging, mining, and hydroelectric development at high elevation lakes, is a concern when considering sustainability of wolverine populations. Road building and increased human traffic, increased access for trappers, and disturbance of high elevation areas used by reproductive female wolverines all have the potential for changing the number and distribution of wolverines in SE AK, not only locally where these resource developments occur, but over a wider area because of the restricted dispersal potential of wolverines in SE AK. Cumulative effects of resource development on wolverine populations in SE AK should be the major focus of wolverine conservation efforts in the region. Monitoring wolverine populations before resource development occurs would provide information on the effects of resource development and on potential mitigation of negative effects.

LONG-TERM MONITORING OF WOLVERINES

The unique ventral pattern on wolverines and the species' territorial social system, tendency toward natal philopatry among the female segment of the population, and scavenging lifestyle make them an ideal candidate for using baited camera stations to monitor changes in important population parameters such as reproduction and survival, sex ratio, and emigration/immigration, particularly when combined with genetic data from hairs collected at the camera stations. Wolverines have a social system based on intrasexual territoriality in which females defend exclusive territories and territories of males overlap those of several females. Recent research (M. Aronsson, Grimsö Wildlife Research Station, Sweden, unpublished data) has demonstrated that, at least in saturated populations, female territories are fairly stable over time, resident females have relatively high fidelity to their territories, and territories that are vacated by death or movement of

the resident female are quickly reoccupied, often by daughters or other related females. This system of spatial structuring of the female segment of the population not only influences the social dynamics but also the population dynamics of local populations including reproduction rate. While female territory size and placement are thought to be influenced to a large degree by food resources, male territories are influenced by the number and distribution of female territories. Therefore, a system designed primarily to monitor female territories will provide information on the male segment of the population as well. Consider that, although we had 44 C&H stations deployed in our study area over the 3 years of the study and detected 28 individual wolverines in that time period, just 9 C&H stations would have detected all these individuals had we known which stations would be the most productive before we began the study. In a hair-snag study of wolverines in Alberta, a similar pattern of visitation to certain hair-snag stations by both male and female wolverines indicated that a long-term monitoring project could be designed around a limited number of C&H stations properly placed. Suitable wolverine habitat for the reproductive segment of a wolverine population will have consistent, predictable resources needed for reproduction and, except for moderately or heavily-harvested populations, will contain saturated populations with stable female territories over time. It should be possible to identify these areas and establish centers of activity of territorial females with a broad array of camera stations and, within 2–3 years, reduce the number of camera stations needed to track changes in the population, concentrating cameras in the territories of the reproductive females. A harvested population of wolverines should show a higher turnover rate, a different sex and age ratio than that of resident animals, and a lower proportion of lactating females than adjacent untrapped populations.

Our integrated system of motion-detection cameras and hair snags provides a cost-effective way of obtaining information on individual ID, sex, genotype, familial relationships, large-scale movement patterns based on DNA, survival rates in multi-year studies, lactation in females, reproductive status of males, turnover rate of reproductive females, centers of activity of individuals, population density using capture-recapture models, and behavioral information such as association of individuals based on the date and time stamp on photographs. Moreover, the technique substantially reduces the costs

of genetic analysis of the wolverines visiting the C&H stations. Selection of monitoring sites requires consideration of habitat characteristics including human developments, wolverine harvest history, and access to the sites, in addition to well-defined objectives for the monitoring effort and means of evaluating the results. We recommend the following protocol for establishing monitoring sites once the purpose of the monitoring effort has been defined and methods for data management and analysis have been established.

Selected C&H stations should be set up and prebaited the first winter to “prime” the stations for the following camera-trapping season. Results of the camera-trapping project near Petersburg demonstrated that wolverines remember the location of food sources and will return repeatedly to these sites even when baits have not been available for months, possibly years for long-term residents. However, it sometimes takes wolverines a long time, even as much as a whole winter season, to locate a source of food within its home range. By prebaiting the stations before starting the first season of camera-trapping, encounter histories at camera stations for resident wolverines will not be biased by the time it takes to set up the stations across the study area or for the wolverines to find the baited stations. Moreover, C&H stations unknowingly placed near sites that already attract resident wolverines (e.g., near avalanche paths where they have found carcasses in the past) will not be more likely to be encountered in the first year of monitoring than in subsequent years.

At the beginning of the monitoring effort, cameras should be placed at a density of at least 1 C&H station/100 km². This density may not be sufficient to locate all female territories in the study area, but the goal is to sample a proportion of the female territories distributed as evenly as possible over the study area. For a dense population, camera stations as close as 7 km may be necessary to detect all resident females (Mulders et al. 2007). The C&H stations should be run from November to the end of April for the first 2 or 3 years to determine the proportion of dispersers that may be in the monitored population during the peak of dispersal in March and April. At least 2 camera-trapping seasons, but more likely 3, will be necessary to identify centers of activity of resident females because wolverines do not necessarily lactate every season and often skip one or more years between reproductions. After the third season, the camera sites that produced

the highest return in photographs of lactating females should be selected for long-term monitoring. The monitoring season can then be shortened to include only the months of March and April when females are lactating, although a short, early winter camera-trapping session may be useful for identifying current year offspring of the resident female.

COMPLETION OF PROJECT OBJECTIVES

All objectives were met:

1. We provided the first information on wolverine movements, home range, and denning habitat in a coastal ecosystem.
2. We used digital motion-detection cameras to gather additional information on the distribution and numbers of wolverines in the study area.
3. We captured hair from photographed wolverines and validated the effectiveness of photo-IDs in establishing the identity and sex of wolverines.
4. We derived a population estimate for wolverines in the study area in 2008 using a new spatial capture–recapture model.
5. We collected tissue samples from harvested wolverines across SE AK, as well as hair from live-trapped wolverines and wolverines visiting camera sites, and through the University of New Mexico provided an analysis of wolverine population structure and interchange with adjacent regions.
6. We evaluated and refined an integrated camera and hair snag (C&H) technique as a method for estimating wolverine distribution and population density, as well as recommended a targeted protocol for monitoring wolverines over the long term.

DELIVERABLES

- 1) Final report to funders; deadline 28 February 2010.
- 2) Publication of a paper on the integrated camera and hair snag technique for monitoring wolverines; submitted on 2 February 2010 to the *Journal of Wildlife Management*; current status *in review*.

- 3) Publication of a paper on the spatial capture–recapture model used for estimating density in our study area; submitted 18 February 2010 to the *Journal of Wildlife Management*; current status *in review*.
- 4) Publication of a paper on an open population model for estimating wolverine density and survival for long-term monitoring projects; submission deadline set for 31 October 2010.
- 5) Publication on genetics of wolverines in Southeast Alaska; submission deadline 31 October 2010.
- 6) Detailed manual on monitoring wolverines with C&H stations; publication of the manual set for 31 October 2010; distribution through The Wolverine Foundation, Inc. and Wildlife Conservation Society Canada.
- 7) Publication of a paper on maintaining sustainable populations of female wolverines in harvested populations; submission deadline set for 2011.

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Table 1. Names and sex of wolverines, detected by either live-trapping (L) or photographing (P), in the study area on the mainland of Southeast Alaska near Petersburg from 2007–2009. All live-trapped wolverines were also photographed in the study area except *Dave* and *Lucky*.

Wolverine	Sex	2007	2008	2009
<i>Cean</i>	F	L	P	P
<i>Cowgirl</i>	F		P	P
<i>Glacier</i>	F			P
<i>Mariner</i>	F		L	P
<i>River</i>	F		P	P
<i>Sarge</i>	F		P	P
<i>Skinny</i>	F		P	P
<i>Snowstorm</i>	F		L	
<i>Sunshine</i>	F		L	
<i>Zigzag</i>	F	P	P	P
<i>Baird</i>	M		P	P
<i>Beaverdam</i>	M	P	L	P
<i>Beebee</i>	M			P
<i>Big Spot</i>	M			P
<i>Castor</i>	M			P
<i>Dave</i>	M	L		
<i>Hoot</i>	M	P	P	
<i>Iceberg</i>	M		P	
<i>Little Spot</i>	M		P	P
<i>Lucky</i>	M	L		
<i>Moonshine</i>	M	L	L	P
<i>Ninja</i>	M			P
<i>Paleface*</i>	M	P	P	P
<i>Pinky</i>	M	P	P	P
<i>Chuck</i>	U		P	
<i>Morse</i>	U		P	P
<i>Splash</i>	U		P	
<i>Splish</i>	U		P	
*Sex not verified but this wolverine occupied the home ranges of two different reproductive females for 3 years so he is assumed to be a male.				

Table 2. Comparison of the identity and sex of wolverines from photographs and DNA collected at camera & hair-snag (C&H) stations on the mainland of Southeast Alaska near Petersburg in 2009. The collection number includes the C&H station number (e.g., C5) and the date the hair samples were collected (mm,dd,yy). The DNA ID column includes the sex and ID number for each individual. The photograph ID is the corresponding name given to each individual. The right-hand column is the sex of the individual identified from photographs.

Collection	# samples	DNA ID	Photograph ID	Sex
C5_040909	3	F3	River	F
C6_050209	6	M1	Pinky	M
C7_042209	2	poor DNA	Pinky	M
C9_040209	5	M11	Moonshine	M
C9_041809	1	M1	Pinky	M
C10_040209	8	M2	Baird	M
C10_041809	3	M1	Pinky	M
C10_050209	12	M2	Baird	M
C12_041909	3	F3	River	F
C13_041809	1	M1	Pinky	M
C13_051809	9	M9	Castor	M
C13_061209	2	poor DNA	Zigzag	F
C14_040209	2	M1	Pinky	M
C20_040309	3	M2	Baird	M
C20_042509	2	M2	Baird	M
C20_051009	5	M2	Baird	M
C21_051009	4	M12	Beebee	M
C22_061009	10	F6	Cean	F
C22_062009	7	F6	Cean	F
C23_033109	2	M10	Little Spot	M
C23_041309	4	poor DNA	Little Spot	M
C24_041309	8	F3	River	F
C24_042509	1	poor DNA	River	F
C25_040309	13	F7	Sarge	F
C25_041409	5	F5	Mariner	F
C25_042509	4	F5	Mariner	F
C25_051809	8	poor DNA	Mariner	F
C27_051809	1	poor DNA	Zigzag	F
C33_040309	5	F4	Glacier	F
C33_042509	5	F4	Glacier	F
C36_042509	4	M8	Beaverdam	M
C36_042509	1	F7	Sarge	F
C36_051809	4	M8	Beaverdam	M
C39_041309	8	M13	Big Spot	M
C39_042509	1	poor DNA	Big Spot	M

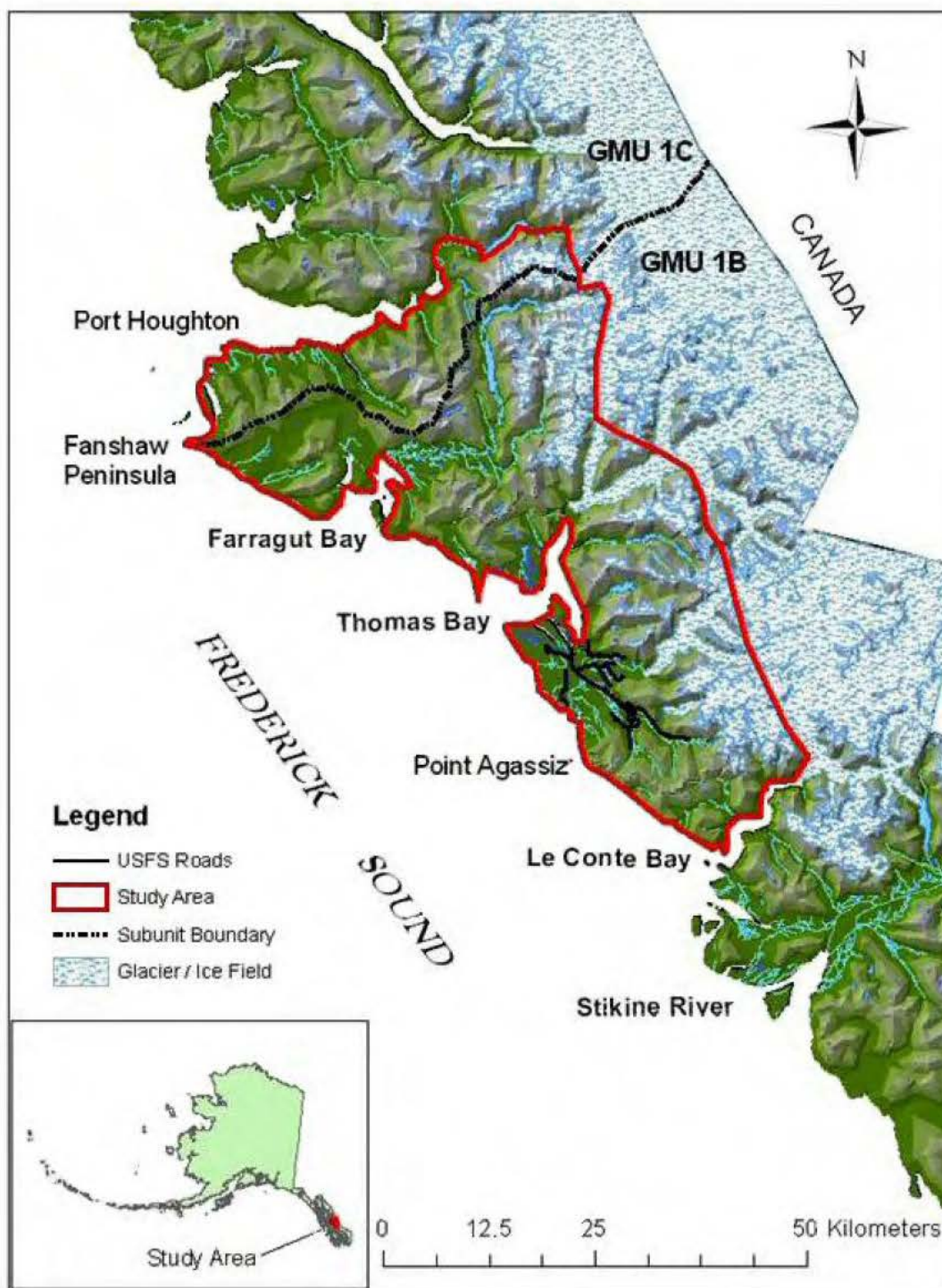


Figure 1. Wolverine study area from 2007-2008 on the mainland of Southeast Alaska near Petersburg, showing locations of Game Management Units 1B and 1C.



Figure 2. Wolverine standing in front of a wooden live trap on the mainland of Southeast Alaska near Petersburg in 2007.



Figure 3. Rolled leather collar with Argos satellite/VHF transmitters on a male wolverine captured on the mainland of Southeast Alaska near Petersburg in 2007.



Figure 4. Size of teats on a female wolverine on the mainland of Southeast Alaska near Petersburg in January 2007, before she had kits (top photo) and in May 2009, while she was lactating (bottom photo).



Figure 5. Abdominal area of a hand-reared male wolverine (top) showing size of testes of the male as a yearling (bottom left) and as a 2-year-old (top and bottom right).



Figure 6. Ventral pattern of a male wolverine captured in a live trap on the mainland of Southeast Alaska near Petersburg in 2008.



Figure 7. An adult female wolverine photographed in an upright position as she was recovering from the tranquilizing drug after being captured in a live trap on the mainland of Southeast Alaska near Petersburg in 2007.



Figure 8. Set up of run poles in 2008 for photographing ventral patterns (a) and in 2009 for photographing ventral patterns and collecting hair (b) from wolverines on the mainland of Southeast Alaska near Petersburg.



Figure 9. Hair-snagging structure made of UHMW black plastic and steel alligator clips mounted on run poles for an integrated system of photographing wolverines while simultaneously collecting hair for DNA analysis in a study of wolverines in 2009 on the mainland of Southeast Alaska near Petersburg.



Figure 10. Snag posts on a prototype of a hair-snagging structure at the end of a run pole showing 5 trigger clips on each snag post. All trigger clips are held open by base clips inserted into the plastic snag posts.



Figure 11. Wolverine hair in a trigger clip collected at a camera & hair snag station in 2009 on the mainland of Southeast Alaska near Petersburg. The trigger clips are attached to a snag post using lanyards and spring clips, which are snapped onto eye screws inserted into the snag posts.



Figure 12. Wolverines on the mainland of Southeast Alaska near Petersburg in 2009 before (top left) and after (top right) dislodging trigger clips on the snag posts of a run pole set up to obtain photographs of ventral patterns while simultaneously collecting hair for DNA analysis. The trigger clips are attached to the snag posts with a lanyard and spring clip attached to eye screws in the snag posts (bottom left). The base clips (bottom middle) are mounted onto the snag posts where they hold the trigger clips (bottom right) open.



Figure 13. Identifying sex of wolverines from photographs taken by motion-detection cameras on the mainland of Southeast Alaska near Petersburg in 2009. Testes may be difficult to see in photographs of yearling male wolverines.



Figure 14. Injuries to a male wolverine (*Lucky*) live-trapped on the mainland of Southeast Alaska near Petersburg in 2007, including blindness in the right eye. Some wounds are fresh and others have healed. This wolverine also had many porcupine quills in its face when it was captured. Wounds of different degrees were common on the wolverines in the study area, but only 2 other male wolverines (*Beaverdam* and *Pinky*) had wounds this serious. All 3 males appeared to be floaters in the wolverine population in the study area.



Figure 15. Drops of milk from the mammary gland of a lactating female wolverine live-trapped in March 2008, on the mainland of Southeast Alaska near Petersburg.

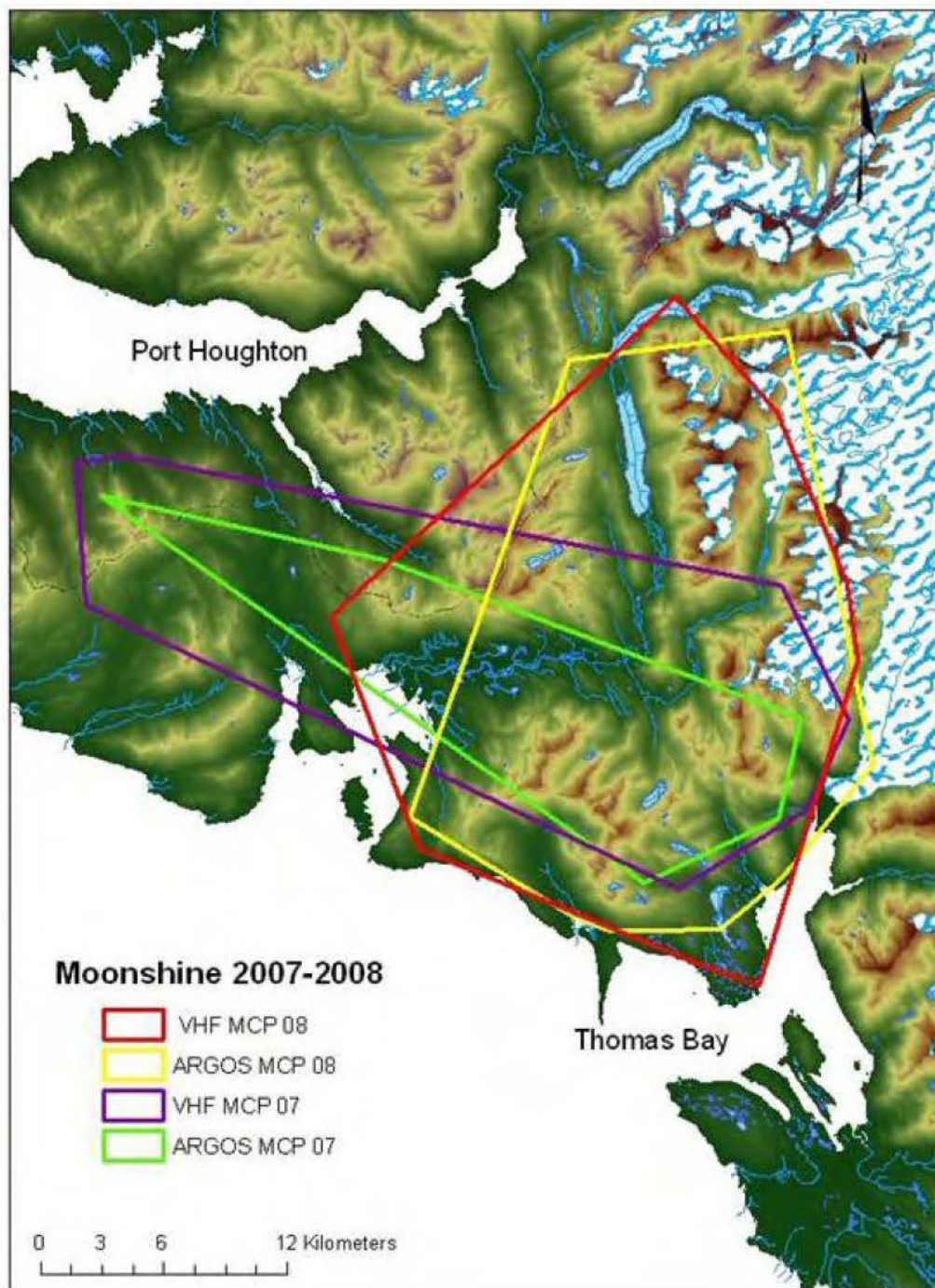


Figure 16. Home range boundaries (Argos and VHF) of male wolverine *Moonshine* in 2007 and 2008 on the mainland of Southeast Alaska near Petersburg. This male was also photographed in the area in 2009.

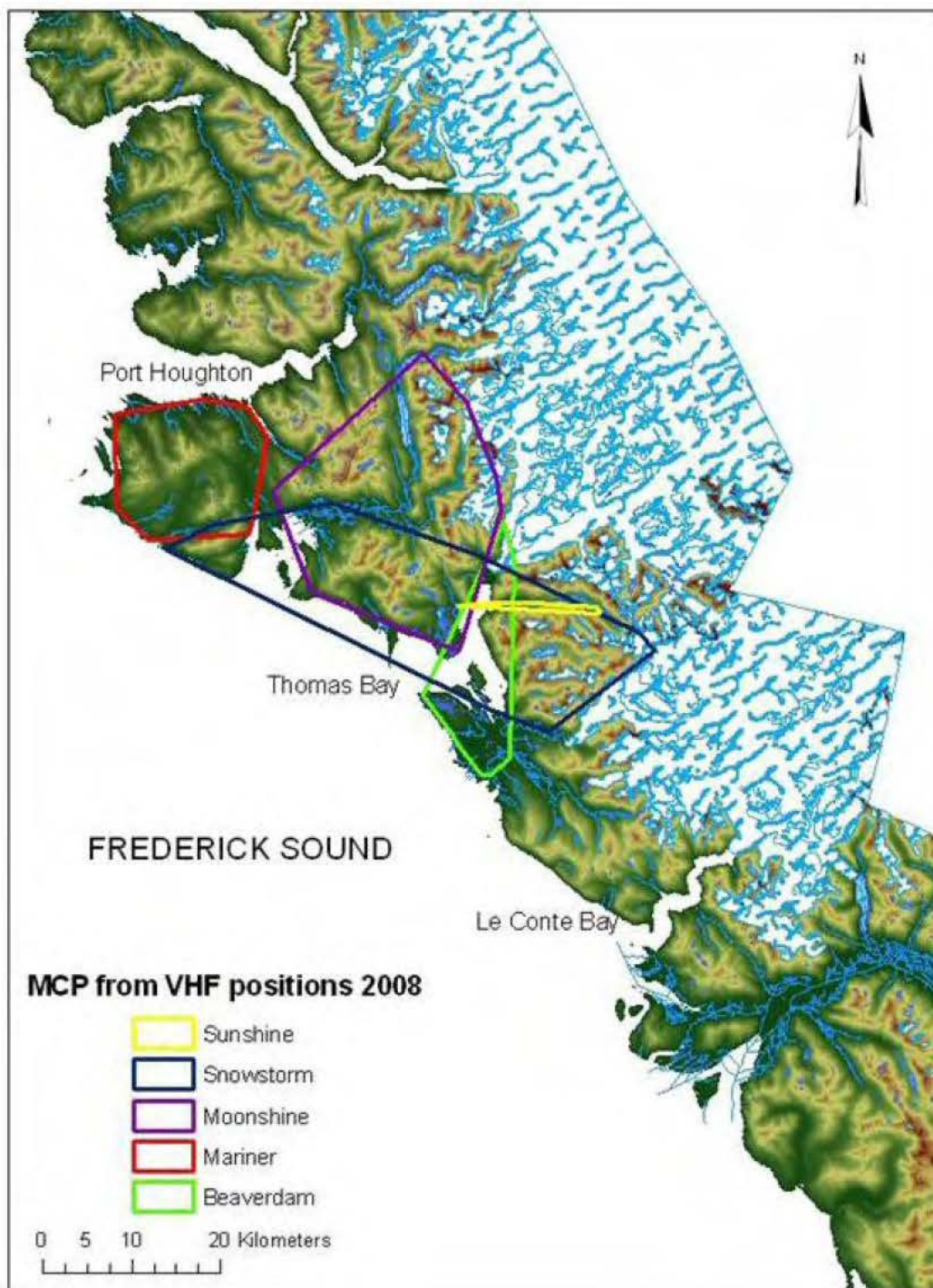


Figure 17. Home range boundaries of wolverines collared in 2008 on the mainland of Southeast Alaska near Petersburg. *Moonshine* and *Beaverdam* were males.

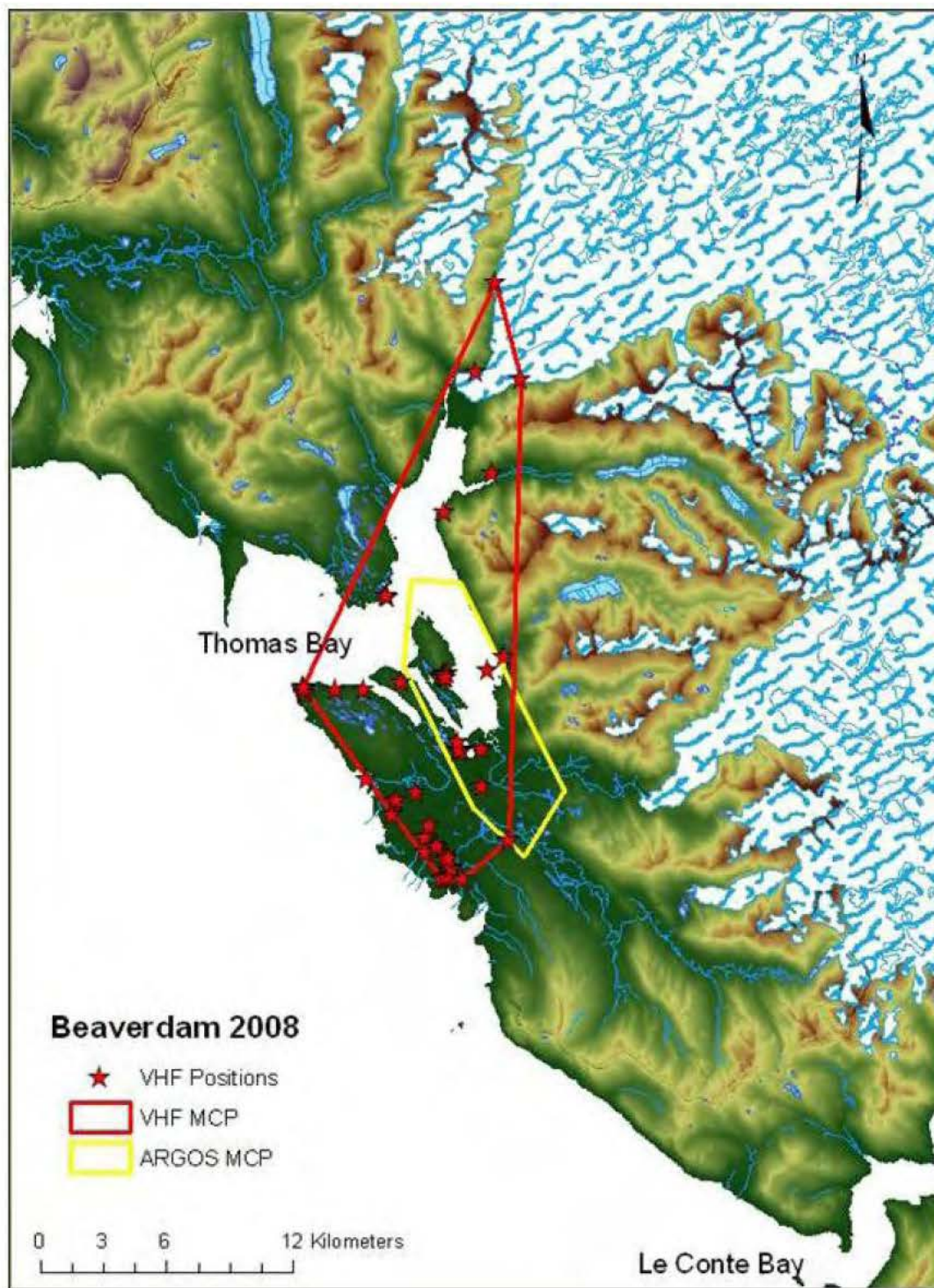


Figure 18. Home range boundaries of male wolverine *Beaverdam* in 2008 on the mainland of Southeast Alaska near Petersburg.



Figure 19. Wounds on the back and head of male wolverine *Beaverdam* at the time of his capture on 3 April 2008 on the mainland of Southeast Alaska near Petersburg; much of the hair was gone from the center of its back where there were several bite marks. The long cable is the antenna for the VHF transmitter.

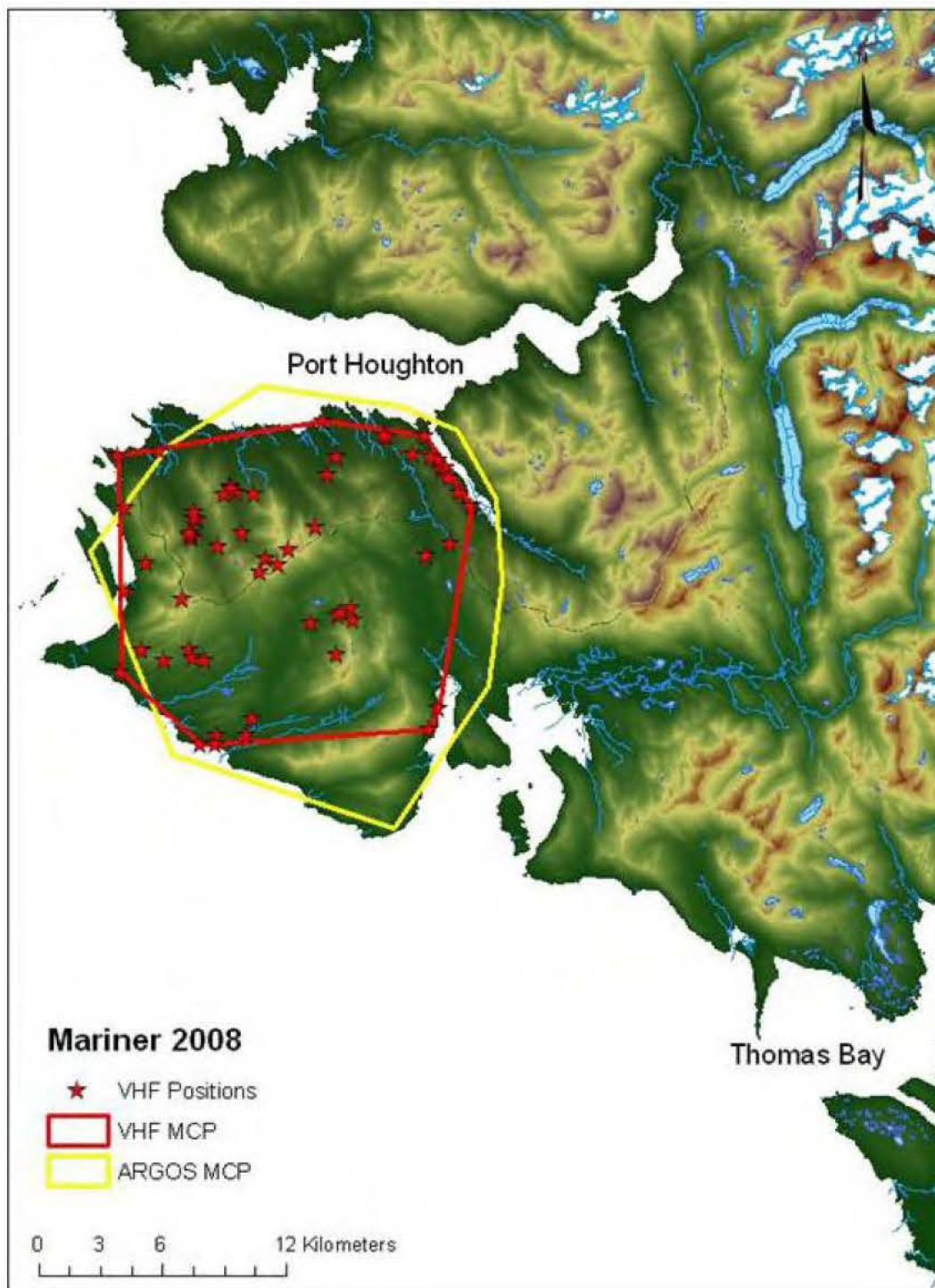


Figure 20. Home range boundaries of parous female *Mariner* in 2008 on the mainland of Southeast Alaska near Petersburg.

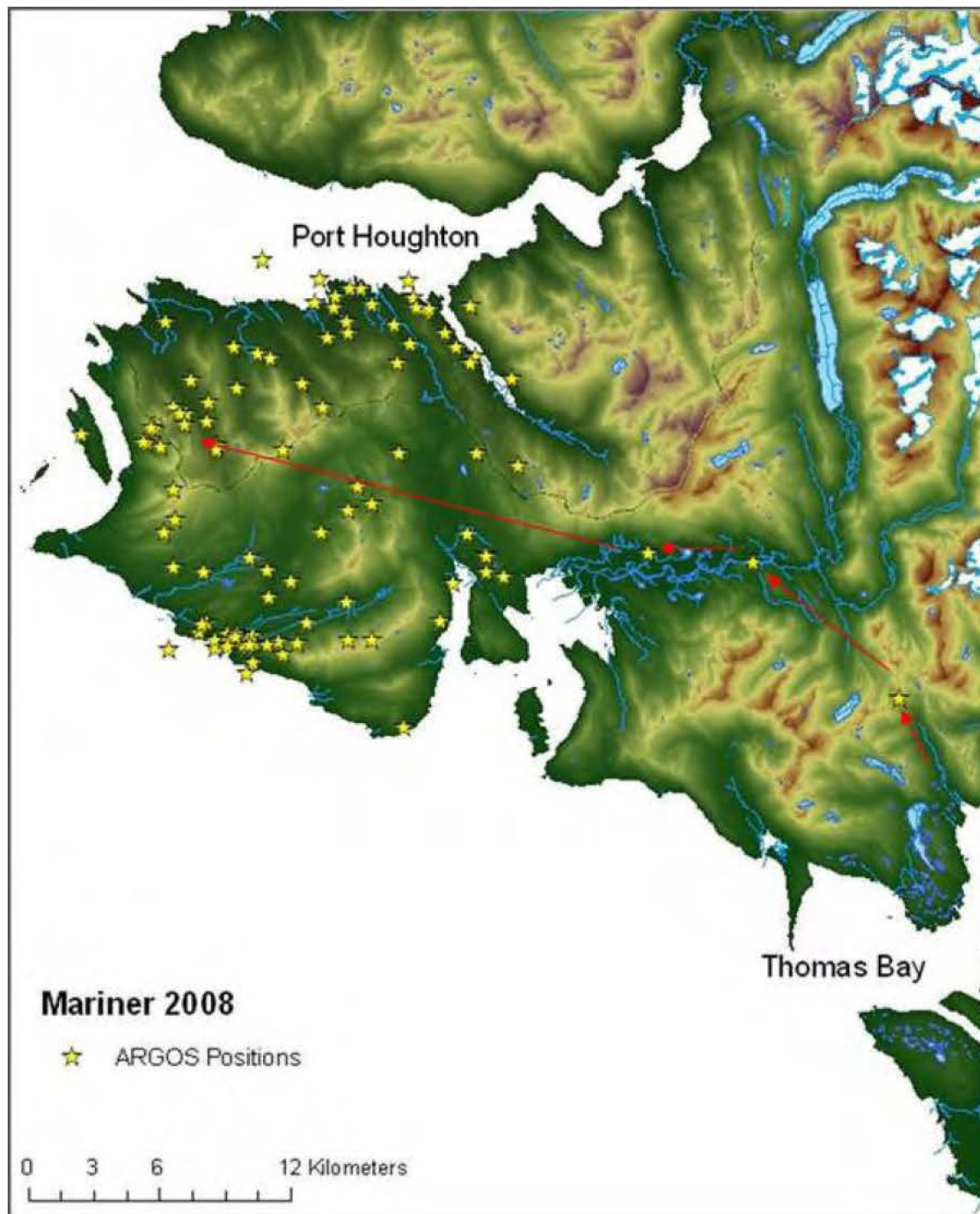


Figure 21. Argos locations of parous female *Mariner* and the direction of movement from her capture site to her established home range in 2008 on the mainland of Southeast Alaska near Petersburg.



Figure 22. Home range boundaries of non-territorial female *Snowstorm* in 2008 on the mainland of Southeast Alaska near Petersburg.

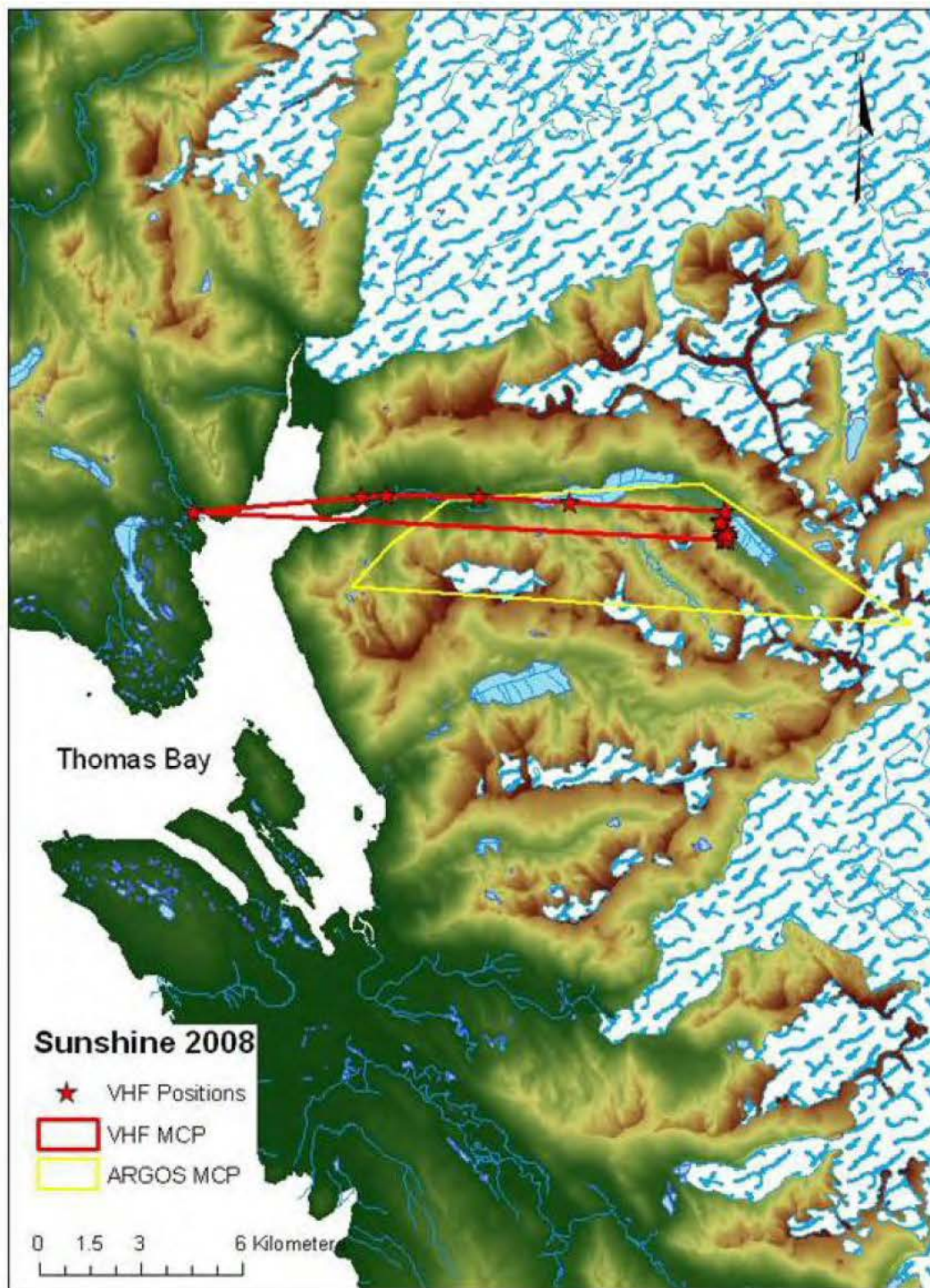


Figure 23. Center of activity for denning female *Sunshine* in 2008 on the mainland of Southeast Alaska near Petersburg.

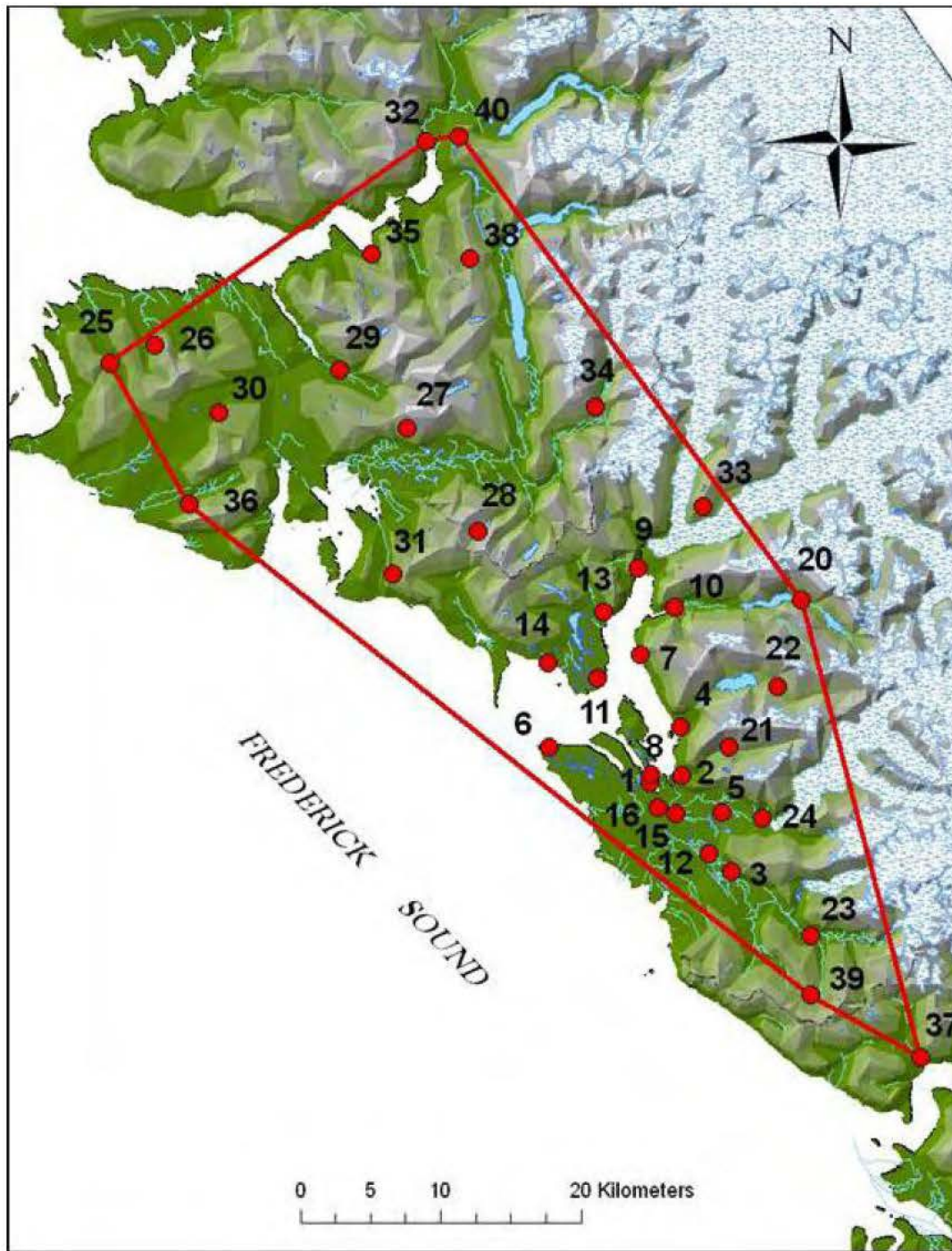


Figure 24. Location of camera stations on the mainland of Southeast Alaska near Petersburg in 2008, showing the number of each camera station and the minimum convex polygon of the camera array.

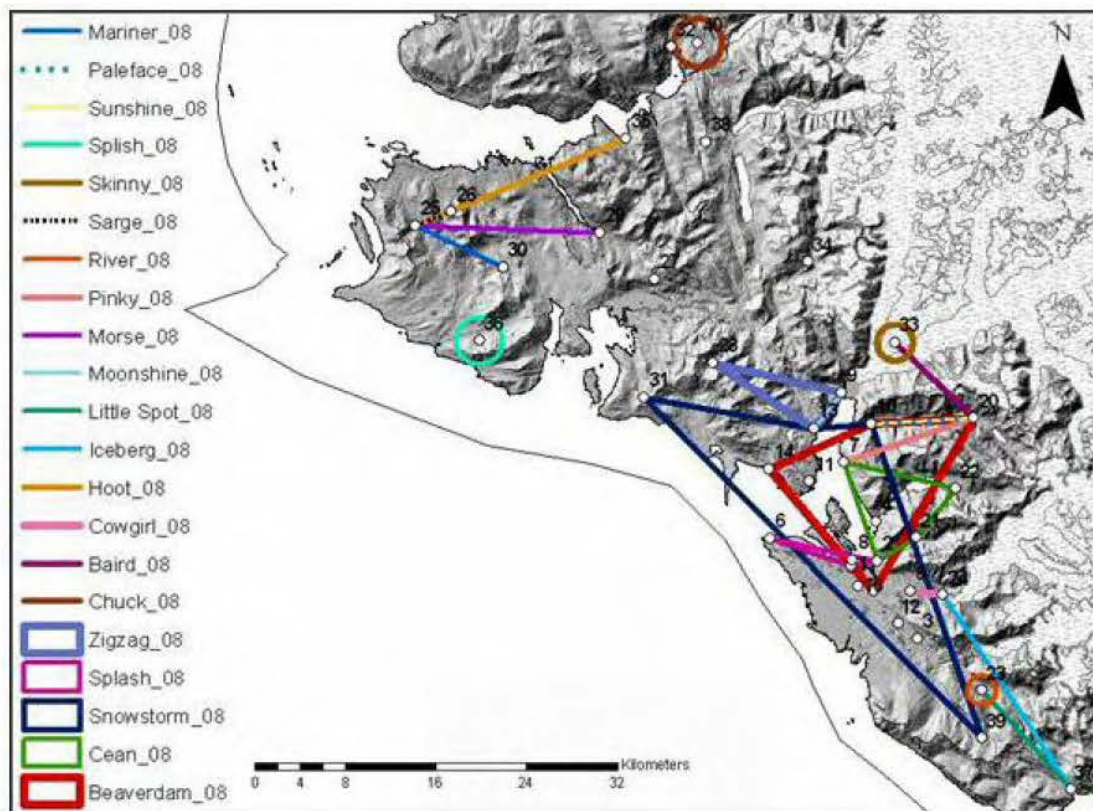


Figure 25. Location of the individual wolverines photographed on the motion-detection cameras on the mainland of Southeast Alaska near Petersburg in 2008. Lines connect camera locations where the same individual was detected; circles indicate individuals detected at only one camera station.

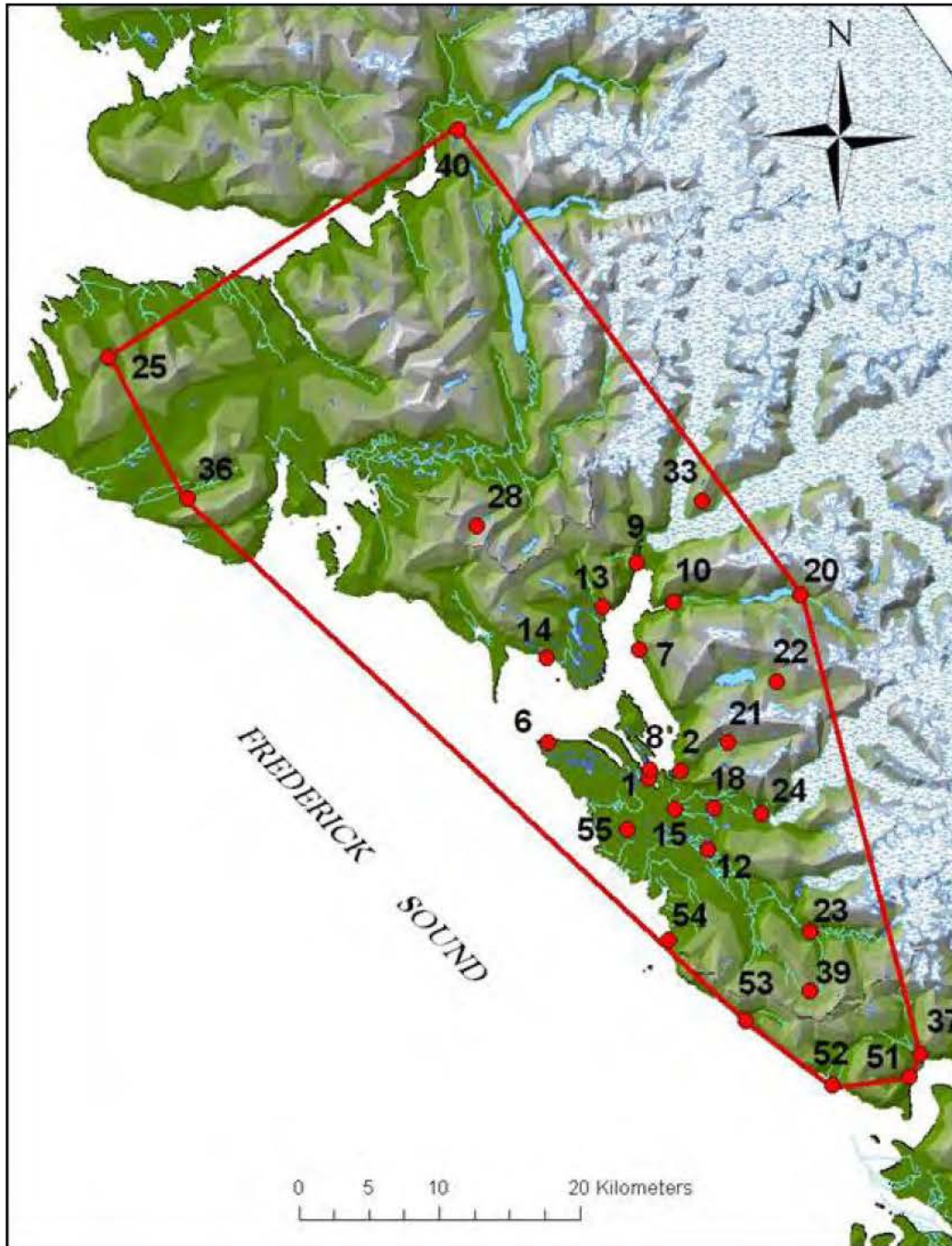


Figure 26. Location of camera stations on the mainland of Southeast Alaska near Petersburg in 2009, showing the number of each camera station and the minimum convex polygon of the camera array.

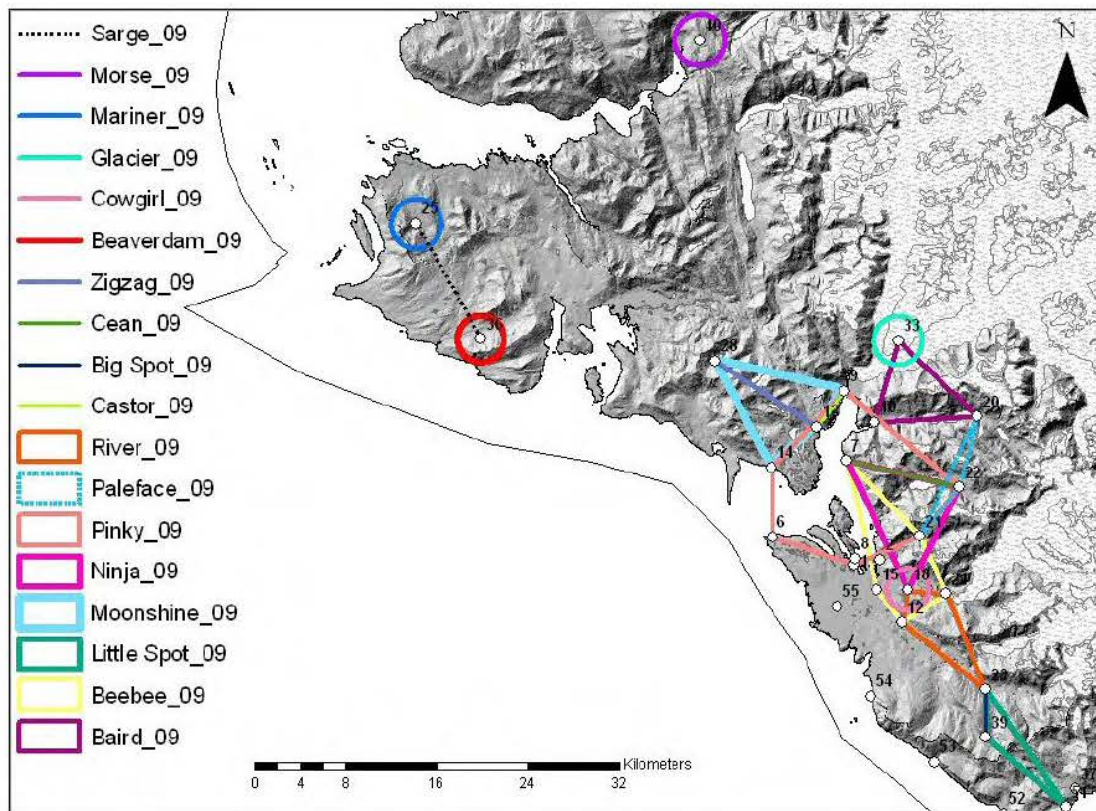


Figure 27. Location of the individual wolverines photographed on the motion-detection cameras on the mainland of Southeast Alaska near Petersburg in 2009. Lines connect camera locations where the same individual was detected; circles indicate individuals detected at only one camera station.

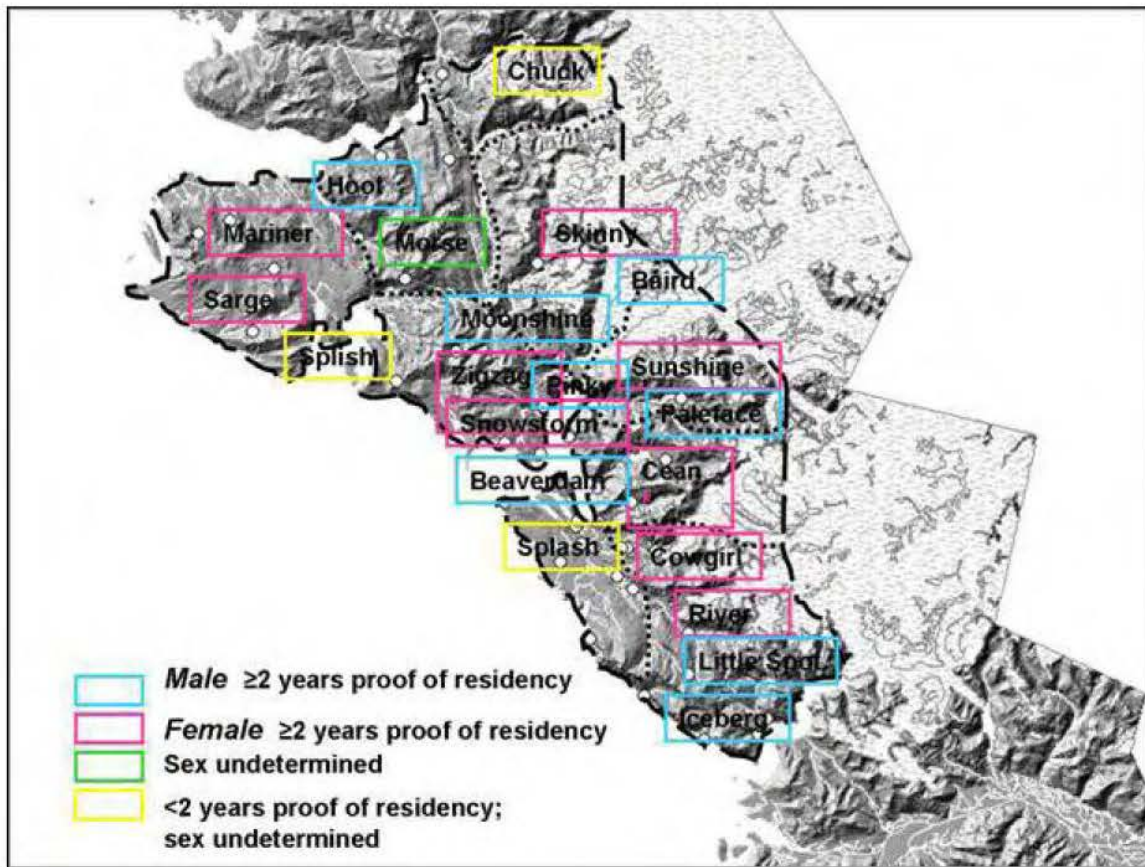


Figure 28. Approximate centers of activity for wolverines photographed in 2008 on the mainland of Southeast Alaska near Petersburg. Number of camera operation days was lower in the northern portion of the study area.

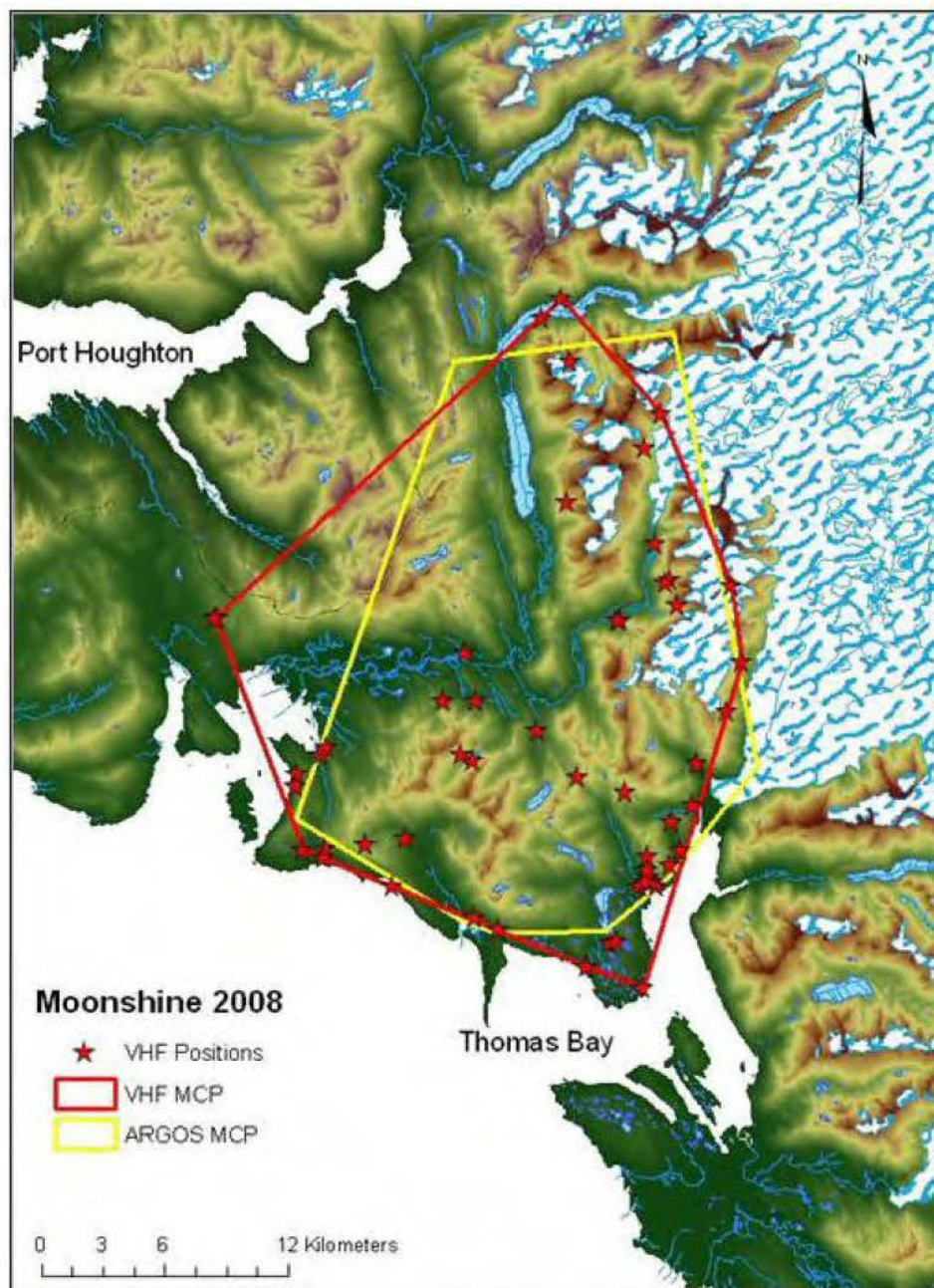


Figure 29. Home range boundaries for adult male wolverine *Moonshine* in 2008 on the mainland of Southeast Alaska near Petersburg. The VHF locations probably overestimated his 100% MCP home range because it contained a large area in the northwest where he was not located.

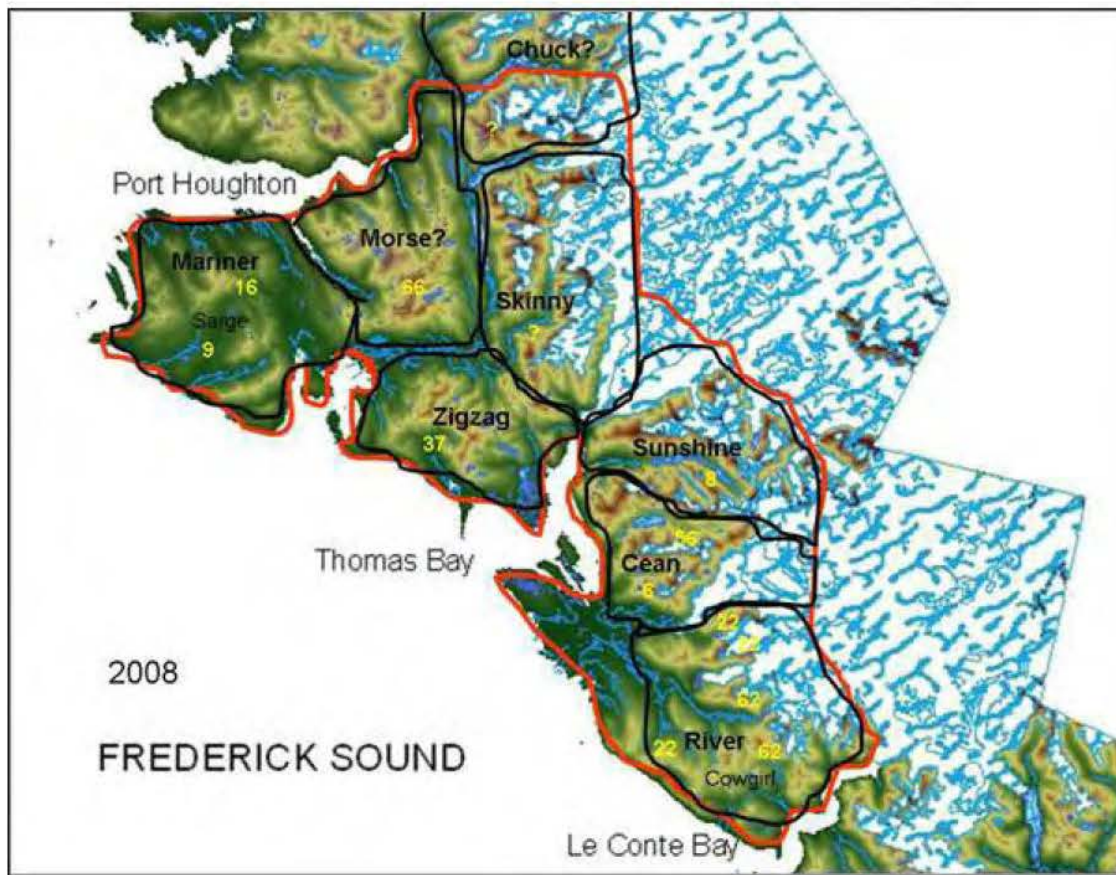


Figure 30. Putative territories of reproductive females in the study area in 2008, based on radiotelemetry, camera data, and spatial patterns of territorial female wolverines. The wolverine *Morse* was probably a male because he was photographed at some of the same camera stations where *Mariner* and *Chuck* were photographed in 2008. The wolverine *Chuck* was photographed only in 2008 and sex was not determined. The pairs *Mariner/Sarge* and *River/Cowgirl* were probably mother/daughter pairs, although it is possible that *River* and *Cowgirl* were both resident females with *River* restricted to the northern half of the delineated range and *Cowgirl* to the southern half. Large portions of their delineated range was in a wilderness area, which we could not access with helicopter so there were no camera stations in the eastern half of the area except on the shore of Le Conte Bay. Yellow numbers indicate the number of mountain goats sighted during a 2005 aerial survey (Alaska Department of Fish and Game, unpublished data), indicating that food resources may have been more abundant in this area than in other portions of the study area.

Appendix A. Manuscript submitted to *Journal of Wildlife Management* on 18 February 2010 describing a spatial hierarchical model for determining density of wolverines using camera-trapping data collected on the mainland of Southeast Alaska near Petersburg in 2008.

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8 RH: Royle et al. • Estimating Wolverine Density

9 **Density Estimation in a Wolverine Population using Spatial Capture–Recapture Models**

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20 **ABSTRACT** Classical closed-population capture-recapture models do not accommodate the
21 spatial information inherent in encounter history data obtained from camera-trapping studies. As
22 a result, individual heterogeneity in encounter probability is induced, and it is not possible to
23 estimate density objectively because trap arrays do not have a well-defined sample area. We
24 applied newly-developed, capture-recapture models that accommodate the spatial attribute
25 inherent in capture-recapture data to a population of wolverines (*Gulo gulo*) in Southeast Alaska
26 in 2008. We used camera-trapping data collected from 37 cameras in a 2,140-km² area of
27 forested and open habitats largely enclosed by ocean and glacial icefields. We detected 21

unique individuals a total of 115 times. Wolverines exhibited a strong positive trap response, with an increased tendency to revisit previously-visited traps. Under the trap-response model, we estimated wolverine density at 9.7 individuals/1,000 km² (95% Bayesian confidence interval: 5.9–15.0). The model provides a formal statistical framework for estimating density from wolverine camera-trapping studies that accounts for a behavioral response due to baited traps. Further, the model-based estimator does not have strict requirements about the spatial configuration of traps or length of trapping sessions, providing considerable operational flexibility in the development of field studies.

KEY WORDS Bayesian, capture-recapture, density, *Gulo gulo*, motion-detection cameras, spatial models, wolverine

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The wolverine is an elusive species that occurs at low densities (Lofroth and Krebs 2007), maintains relatively large home ranges (Persson et al. 2009), and at times undertakes very long movements (Inman et al. 2004). For these reasons, estimating density for wolverines has been problematic and early attempts lacked measures of accuracy and precision (review by Lofroth and Krebs 2007). More recently, Golden et al. (2007) estimated wolverine density based on aerial track surveys in winter in open habitats. Mulders et al. (2007) and Fisher et al. (2009) used DNA from hairs collected at bait sites to estimate wolverine density. Lofroth and Krebs (2007) used capture–recapture estimates of density from live-trapped wolverines and were the first to publish density estimates using photographs of wolverines captured on motion-detection cameras. None of the methods above that used capture–recapture approaches considered the spatial context of the data.

Closed population models are widely used to estimate abundance or density of animal populations from fixed arrays of traps or other devices that obtain information on encounter history (Borchers et al. 2002). Direct interpretation of population density obtained from such models is difficult because the sampled area of trap arrays is not a well-defined concept in the sense that, while one may delineate a geographic area for the placement of traps, it is not usually possible to delineate the effective sampled area (i.e., that area from which captured individuals are drawn). This problem has long been recognized in the literature (e.g., Dice 1938, Stickel 1954, Wilson and Anderson 1985, Efford 2004). Authors have used different approaches to resolve the problem of defining the effective area, most often drawing polygons around the trapping arrays, buffering, and adjusting estimates of population size obtained from closed population models (Karanth and Nichols 1998). The problem with this approach is that it is ad hoc. Rules for buffering (e.g., mean maximum distance moved) are arbitrary, not consistent among studies, and such approaches induce uncertainty into estimates of density that have not been accommodated formally into density estimation. Moreover, the spatial organization of traps within the resulting buffered area suggests heterogeneity in encounter probabilities among individuals, which this method does not formally accommodate.

Recent developments of spatial capture–recapture (SCR) models (Efford 2004, Borchers and Efford 2008, Royle and Young 2008) provide a formal method for inference from capture–recapture studies by allowing for estimation of density. These models extend standard closed population models (e.g., Otis et al. 1978, Borchers et al. 2002, Lukacs and Burnham 2005) to include a spatially explicit model for the distribution of individuals in space. These models are spatially explicit in the sense that they are parameterized in terms of a spatially-indexed, individual random effect. The trap-specific encounter probabilities are modeled as a function of

distance between traps and individual activity centers. These models have proved useful for estimating density in a number of carnivore sampling problems including bears (Gardner et al. 2010), tigers (Royle et al. 2009a,b), and small cats (Gardner et al. in press; Kéry et al. in press). Our objective was to develop a spatially explicit capture–recapture model for estimating density using data from a camera-trapping study of wolverines (*Gulo gulo*) in Southeast Alaska.

STUDY AREA

The study area comprised 2,140 km² of coastal tidelands, temperate rainforest, muskeg, alpine habitats, and glaciers in the Tongass National Forest on the mainland of Southeast Alaska (Fig. 1) near Petersburg (56 ° 48.67602 ' N, -132 ° 57.07452 ' W). Elevation in the study area ranged from sea level to 2,164 m. Temperatures at sea level during the camera-trapping sessions ranged from –4°C to 7°C but were considerably colder at higher elevations, although rarely falling below –23°C. Precipitation (mainly as snow) was very heavy during the study, exceeding 4 m in depth at higher elevations. Sitka spruce (*Picea sitchensis*) or western hemlock (*Tsuga heterophylla*) dominated the overstory on productive forest sites but poorly drained sites supported mountain hemlock (*Tsuga mertensiana*), Alaska-yellow cedar (*Chamaecyparis nootkatensis*), or western red cedar (*Thuja plicata*). The forested habitat where cameras were deployed occurred within 50 km of the coast of Frederick Sound, which bordered the study area on the west. To the north the study area was bordered by Port Houghton, on the south by Le Conte Bay, and on the east by the Stikine Icefield. These geographic barriers limited movements of wolverines into the study area (Frances 2008). Prior to our study, fur trappers targeted wolverines along low-elevation forestry roads and along beaches but, in cooperation with our study, they refrained from setting traps for wolverines. Other than wolverines, the 3 species that

triggered our camera traps most often, in order of frequency in photographs, were American marten (*Martes americana*), bald eagle (*Haliaeetus leucocephalus*), and black bear (*Ursus americanus*).

METHODS

Camera Trapping

Individual wolverines have a unique pattern of light-colored pelage on their ventral surface and this ventral pattern can be used to identify individuals from photographs, much as stripes and spots have been used to identify individual wild cats from photographs (Karanth and Nichols 1998, Soisalo and Cavalcanti 2006, Balme et al. 2009). Technique development with captive wolverines and radio-tagged wild wolverines demonstrated that this pattern remains the same over the lifetime of the individual (A. J. Magoun, WRAM, unpublished data). To photograph the ventral pattern, we lured wolverines to camera traps using remnants of moose (*Alces alces*), deer (*Odocoileus hemionus*), and beaver (*Castor canadensis*) donated by hunters and trappers. We selected trap sites (Fig. 1) based on recommendations of local fur trappers, our knowledge of suitable wolverine habitat, and logistical constraints of accessing mountainous terrain in our study area.

For camera sites accessed by helicopter, we fabricated run poles by mounting a 107-cm-long piece of 2x4 (38 mm x 89 mm) dimensional lumber onto a 25-cm-long piece of 4x6 (89 mm x 140 mm) dimensional lumber (Fig. 2; left panel). For camera sites accessed on the ground, we used either the same type of run pole or one fashioned from logs found on site. We suspended bait from a horizontal cable that was stretched between 2 trees that were 3–4 m apart. We attached a run pole to one of the trees at 1–2 m above ground or snow with the distal end of the run pole extending toward the other tree on which we mounted a digital motion-detection camera

(Trailwatcher®, Monticello, GA). The bait was suspended 0.5–1.0 m above the end of the run pole. To access the bait, a wolverine would have to walk out to the end of the run pole, thereby triggering the camera while the animal was looking up at the bait exposing the ventral pattern (Fig. 2; right panel).

We set cameras to take photographs whenever there was motion in front of the camera. With the flash on all the time, the cameras took photos approximately every 4 seconds when an animal was on the run pole. Each photograph file included the date and time the photograph was taken. We considered each 24-hour period a trapping session. We attempted to visit trap sites at least every 2 weeks to change batteries and flash cards and to rebait the sites, but this was not always possible due to weather and logistical constraints. If cameras were inoperable when we revisited the sites, we used the last day a photograph was taken to calculate the total number of days the camera was operational (i.e., number of trapping sessions).

Statistical Methods

The data from a SCR study consist of the individual and trap-specific encounter histories $y_{i,j,k}$ for individual $i=1,2,\dots,n$; traps $j=1,2,\dots,J$; and sample periods $k=1,2,\dots,K$. In practice, sample periods are daily intervals and thus we obtain a conventional encounter history (i.e., a sequence of 0's and 1's) for each individual and trap. In our study, traps were operational for variable lengths of time. The temporal nature of encounter histories is important when time effects, such as date of the sample or behavioral response, are of interest (Kéry et al. in press; Gardner et al. in press). When explicit temporal effects are not relevant, encounter histories may be summarized by individual and trap-specific frequencies, i.e., $y_{i,j}$, the number of times that individual i was encountered by trap j out of K_j sample periods.

The key idea in SCR models is that they formally relate the encounters of individuals to where these individuals spend time over the trapping period. In particular, if we imagine that an individual occupies a defined area for a given period of time (i.e., a seasonal home range or use area) and its activities are centered in that area for that period, then it is natural to expect that the probability of an individual encountering a trap is a function of the distance between that individual's activity center and the trap. Efford (2004) introduced this concept, which was formalized in a likelihood framework by Borchers and Efford (2008). Royle and Young (2008) developed a Bayesian hierarchical formulation of a similar class of models. Here we assume that encounter probability is related to distance between each activity center and trap according to:

$$p_{i,j} = 1 - \exp(-\lambda_0 \exp(-d_{i,j}^2 / \sigma^2))$$

where $d_{i,j} = \|s_i - x_j\|$ is the distance between activity center of individual i , s_i , and location of trap j , x_j . This specific form of the detection function is justified (Royle et al. 2009b) as “the probability of at least 1” encounter under a Poisson model where the encounter rate declines with distance according to a normal probability kernel with scale parameter σ . To interpret this, suppose that it was feasible to deploy a very dense, regular grid of traps such that individuals are encountered frequently at many locations. Then, under the model, encounter frequencies would resemble a histogram approximation to a bivariate normal density around an individual's activity center. However, in practice we only observe individuals at a small fixed number of traps and thus such a pattern will typically not be evident.

This model for detection probability can be re-expressed as a linear model on the complementary log-log scale:

164 $\text{cloglog}(p_{i,j}) = \log(\lambda_0) - b1 * d_{i,j}^2$
 165 where $b1 = (1/\sigma^2)$. Because the model can be formulated as a binary regression model in this
 166 fashion, useful extensions of the model are straightforward. For example, primarily a scavenger
 167 in winter (Banci 1994), the wolverine has a strong tendency to cache food and to return
 168 repeatedly to a food source to remove the food to caching sites. As such, it is reasonable to
 169 assume that once a wolverine has found a baited camera site, the same wolverine is likely to visit
 170 the site repeatedly. Thus, we will consider the possibility of a behavioral response (referred to as
 171 Model M_b in classical capture-recapture), which for our model involves adding a single
 172 regression term to the model and defining a regression variable, say $x_{i,k} = 1$ if individual i was
 173 captured previous to sample session (day) k . In the context of SCR models, it is natural to
 174 define the behavioral response to be trap-specific (Royle et al. 2009b, Gardner et al. 2010). That
 175 is, $x_{i,j,k} = 1$ if individual i has previously been captured in trap j . Thus, if an individual is captured
 176 in a specific trap, then the probability of subsequent encounter is increased or decreased only for
 177 that trap. We consider this a local behavioral response in contrast to what could be considered a
 178 global behavioral response as in the standard model (Model M_b), i.e., a behavioral response that
 179 does not depend on where the individual was encountered. The latter type of response would be
 180 unrealistic in most spatial sampling situations. Because the covariate is time-dependent, we
 181 require a definition of the model in terms of individual Bernoulli trials, $y_{i,j,k}$, with probability of
 182 encounter $p_{i,j,k}$ with

183

$$184 \quad \text{cloglog}(p_{i,j,k}) = \log(\lambda_0) - b1 * d_{i,j}^2 + b2 * x_{i,j,k}$$

185

Note that distance $d_{i,j}$ is a function of individual activity center s_i , which is an unobserved random effect. The model thus appears very similar to so-called “individual covariate models” (e.g., Royle 2009). Because the individual activity centers are latent variables, we specify a probability distribution for them. The standard assumption (Efford 2004, Borchers and Efford 2008, Royle and Young 2008) is that the s_i are uniformly distributed in space. As such, we note that the model is essentially a standard, closed population model but augmented with a spatial random effect that describes the juxtaposition of individuals with the trap array.

With this additional model assumption for the random effects, the model can be analyzed using methods that are standard for analyzing Generalized Linear Mixed Models (GLMMs). In particular, they are conveniently analyzed by Bayesian methods using a technique known as data augmentation (Royle et al. 2007). Royle and Gardner (2010) and Gardner et al. (2010) implement similar models in WinBUGS (Gilks et al. 1994), and they provide the R and WinBUGS scripts to fit the models. In addition, a native R implementation developed from Royle et al. (2009a,b) provides a more efficient MCMC implementation in some cases. We used these R programs which are now available in the downloadable R package SPACECAP (Singh et al. 2010).

Bayesian analysis proceeds using a simulation-based method (i.e., Markov chain Monte Carlo or MCMC; Link et al. 2002), which requires that we simulate realizations of the underlying point-process. To do this we must specify the region over which points are distributed (called the state-space of the point process), a geographic region that we indicated previously by S. In general this must be specified regardless of whether one adopts a Bayesian or frequentist (e.g., Borchers and Efford 2008) approach to inference. In the latter case, one must integrate the random variables (activity centers) out of the conditional-on- s_i likelihood. When the state-space

of the point process is precisely defined, this in turn defines the parameter N as the number of individual activity centers located within the prescribed state-space. The parameter N will be estimated (see below). Density is a derived parameter $D = N/\text{area}(S)$, where N is the parameter of the model and $\text{area}(S)$ is the known area of the prescribed state-space.

In a Bayesian analysis, the size and configuration of the state-space requires careful consideration because the model is analyzed conditional on the underlying point process. That is, the point process is explicitly simulated, and thus the state-space must be described precisely. There are 2 basic approaches to choosing the state-space. We might consider describing the geographic region containing the traps only where habitat is suitable. Alternatively, we can define a regular polygon (e.g., rectangle) containing the trap array without differentiating unsuitable habitat. Although defining the state-space to be a regular polygon has computational advantages (e.g., we can implement this more efficiently in WinBUGS and cannot for irregular polygons), a regular polygon induces an apparent problem of admitting into the state-space regions that are distinctly non-habitat (e.g., oceans, large lakes, ice fields, etc.). It is difficult to describe complex sets in mathematical terms that can be admitted to this spatial model. As an alternative, we can provide a representation of the state-space as a discrete set of points that will allow specific points to be deleted or not depending on whether they represent habitat. This is primarily necessary to provide control over the geometry and morphology of what we consider to be suitable habitat. In what follows, we have provided an analysis of the camera data using both approaches. In the first case, we defined the state-space to be a regular continuous polygon (a rectangle) but did not clip out non-habitat. To define the continuous state-space we overlaid the trap array (Fig. 1) on a rectangular region extending 40 km beyond the traps in each cardinal direction. In the second set of analyses, we used a discrete representation of the state-space but

then deleted points that were not over land. For that approach we used 2 grid resolutions (2 km and 8 km) before clipping out unsuitable points. Our interest in doing this was to evaluate the relative influence of grid resolution on estimated density because the coarser grids will be more efficient from a computational stand-point and so we would prefer to use them, but not if there is a strong influence on estimated density.

While N is arbitrary in the sense that it necessarily increases with S , which is also arbitrary, we note that the density of points $N/|S|$ is invariant to S as long as S is sufficiently large, which can be verified by conducting a trial MCMC run. We can then address that N is unknown using data augmentation (Royle et al. 2007; Royle 2009), a method for implementing MCMC for capture-recapture models in which N is unknown. We can then produce spatially-explicit density estimates by tallying up, from the posterior samples obtained by MCMC, the frequency of activity centers in any arbitrary polygon within the state-space and standardizing by the relevant area (e.g., Gardner et al. in press).

RESULTS

We operated 37 camera sites (Fig. 1) over a 165-day period during January–May 2008. Cameras were operational for variable periods of time (min = 5 days, max = 108 days, median = 45 days). The cameras captured 21 unique individuals a total of 115 times. Most individuals were captured 1–6 times, with 4, 1, 4, 3, 2, and 1 individuals in each of the 6 categories, respectively. In addition, 1 individual each was captured 9 and 14 times and 2 individuals each were captured 10 and 13 times. The number of unique traps that captured a particular individual ranged from 1–6, with 5, 9, 4, 1, 1, and 1 in each of the 6 categories, respectively, for a total of 50 unique wolverine–trap encounters. Multiple captures of an individual at the same trap explains the difference between total captures and the unique encounters.

The posterior mean density under the continuous state-space model was 5.8 individuals/1,000 km² (Table 1), which can be regarded as a point estimate, and uncertainty in the value can be characterized by the percentiles of the posterior distribution, labeled 2.5% and 97.5% in Table 1. We note that the posterior mean of the detection scale parameter $\sigma = \sqrt{1/b_1}$ is 6.4 km.

For the 2-km and 8-km discrete state-space models (Table 1), posterior summaries of model parameters and related quantities were similar. Densities were 8.6 and 8.2 individuals/1,000 km², respectively, consistent with that fact that the finer resolution grid accommodates higher levels of heterogeneity in detection probability (Link 2003). To improve computational efficiency, we used the 8-km discrete state-space in the development of the behavioral response model (Table 1). Consistent with our expectations, there was a large positive effect of previous capture in a trap and an increase in estimated density under that model (posterior mean: 9.7 individuals/1,000 km²).

DISCUSSION

The model based on the continuous state-space resulted in a much lower density estimate than that based on the discrete grid approximations to the state-space. This is because most of the stated area of the continuous state-space lay over water and thus the model distributed some individual activity centers over water, resulting in a corresponding decrease in apparent density compared with the discrete representation that allowed for water area to be removed. We noted little difference in estimated density and other model parameters between the 2-km and 8-km grids and thus we feel that, as an operational compromise, the relatively coarse grid provides adequate estimates of density.

Integrating the trap identity information explicitly into the model allowed for the consideration of more realistic models of the behavioral response. The intuitively appealing model that we fitted to our data, in which the behavioral response operates on individual traps (what we referred to as a local behavioral response), has not been considered in the standard capture–recapture context. The model without a behavioral effect estimated a density of about 8.2 wolverines/1,000 km² with an estimated detection scale parameter (σ) of 6.8 km. In contrast, the estimated density was 9.7 wolverines/1,000 km² and σ 9.8 km when we included the behavioral response. This strongly positive, trap-specific behavioral response indicated a tendency for individuals to revisit camera traps subsequent to their initial visit, a result that is unsurprising when traps are baited and successive captures are not independent. The apparent encounter probability was artificially high under a behavioral response and was properly adjusted downward when a behavioral response was admitted in the model, yielding a corresponding increase in estimated density.

By using SCR models for estimating wolverine density, we avoided a number of problems inherent in non-spatial models for species that are hard to detect and result in small sample sizes. Logistical constraints of operating in northern wilderness environments in winter usually make it impossible to ensure that the number of trap sessions among camera sites is the same, but trapping sessions of equal length are not a requirement of the SCR models. For non-spatial models it is not at all obvious how to condense such variable trap-specific encounter histories into standard encounter histories that ignore trap identity, therefore, excess recaptures (i.e., wolverines captured multiple times at a unique site in a session) are discarded or pooled in some fashion (e.g., Mulders et al. 2007) resulting in loss of information about the encounter process. An obvious problem with pooling data from a trap array into discrete periods in order to

create non-spatial encounter histories is that it is not obvious how to do this when trap operation is staggered in time and deployed traps are operational for varying lengths of time. Pooling of data was not necessary in our SCR models because they are based on trap-level encounters of individuals.

The SCR models resolve 2 specific problems inherent in the application of non-spatial, closed population capture–recapture models. First, it is not necessary to use ad hoc methods to devise an effective sample area to which the estimated N is applied because, instead, the location and number of individual activity centers (s) are regarded as unknown parameters (random effects) to be estimated. Secondly, SCR models account for individual heterogeneity in encounter probability that is necessarily induced by juxtaposition of individuals with traps. In particular, the model assumes that encounter probability is a function of distance between traps and activity centers, and thus encounter probability decreases to 0 as distance from the trap array increases.

As with all closed population models, the SCR models used here assumes demographic closure, i.e., the absence of permanent additions or removals from the population during the trapping period. The closure assumption in the SCR model is manifest in the static nature of the individual activity centers. While each individual has its own activity center, the model assumes that the activity centers are constant over the period of the trapping period. To the extent that non-closure is due to the presence of transients, such non-closure could perhaps be effectively modeled as an individual-specific encounter probability scale parameter, ϕ , accommodating apparent variability among individuals in home range size. We are currently devising methods for fitting such models. Extensions of the model that allow for mortality and recruitment have also recently been developed (Gardner et al. 2010). In addition to accounting for explicit

violation of the closure assumption due to population dynamics, such models can accommodate non-static activity centers, such as may be necessary in multi-year studies as individual home ranges shift due to resource variability, behavioral interactions, and other biological phenomena.

We also plan further extensions to this basic SCR model by adding covariates that could address other sources of heterogeneity in individual capture probabilities. For example, based on the smaller average size of home ranges for female wolverines (Persson et al. 2009), we expect that the sex of individuals influences the average distances between activity centers and encountered traps for males and females. Sex of wolverines can be determined using camera-trapping techniques (A. Magoun, unpublished data), as well as from DNA, and could easily be incorporated as an individual covariate in our models. Moreover, we suspect that camera traps that are visited by female wolverines, particularly lactating females in March and April, may indicate prime habitat where wolverines are concentrated across the landscape (A. Magoun, unpublished data). Therefore, we suggest a trap-specific covariate that considers visits by females. Finally, Copeland et al. (2010) showed that late spring snow cover may be correlated with suitable wolverine habitat, suggesting another covariate that could be incorporated into the SCR models for study areas that are heterogeneous for spring snow cover.

Management Implications:

The SCR models we developed in this study remove several mathematical and logistical problems that arise in estimating density of wolverines and other cryptic, low-density species. The SCR models can be applied to any capture-recapture technique that provides individual and trap-specific encounter histories. Besides incorporating spatial data and thereby avoiding ad hoc derivations of an effective area, the SCR models permit formal mathematical testing of assumptions regarding capture probabilities. Furthermore, the capability of the SCR models to

incorporate variables that affect encounter probability provides managers the flexibility to modify camera-trapping techniques in ways that improve capture success (e.g., using multiple cameras at trapping stations or adjusting camera activation periods to accommodate species-specific activity). Finally, we believe that methods for estimating density of species are most useful when they accommodate the realities of working under rigorous and unpredictable field conditions on species that are difficult and expensive to study.

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453 *Associate Editor:*
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List of Figures

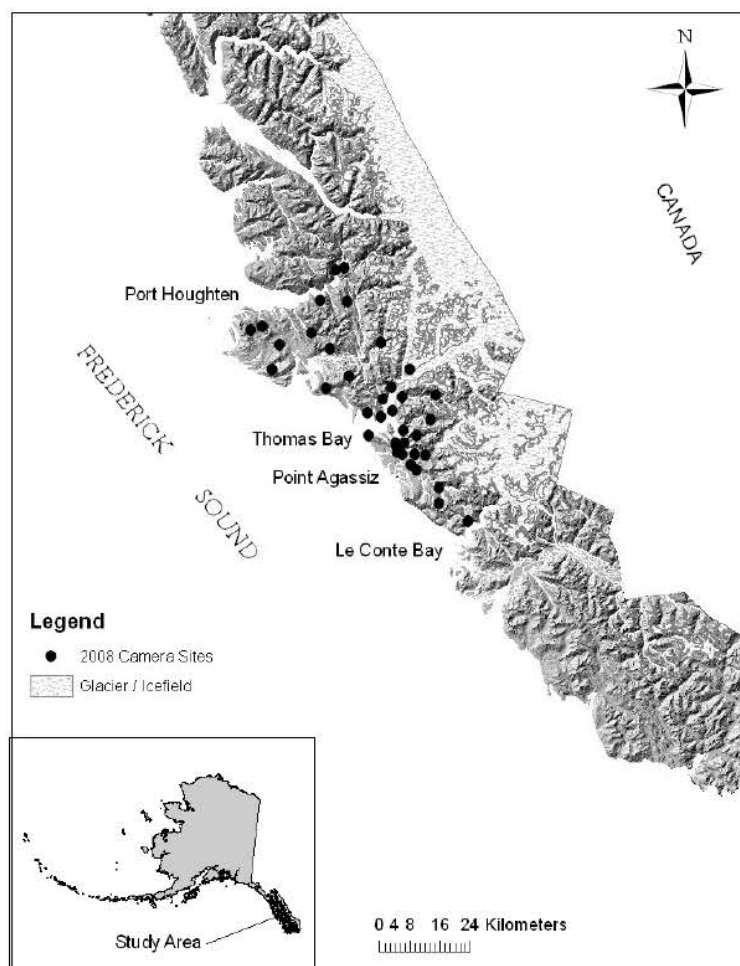
Figure 1. Study area on the mainland of Southeast Alaska near Petersburg showing location of camera & hair snag (C&H) stations (black dots) in 2008 used for detecting wolverines. Stippled area indicates the extent of the Stikine Icefield to the Canadian border. The C&H array is largely surrounded by ocean and icefields except for a 20-km-wide forested area at the head of Port Houghton.

Figure 2. Camera & hair (C&H) snag stations on the mainland of Southeast Alaska near Petersburg in 2008 used for detecting wolverines. The left panel shows the general set up of the run pole, bait, and camera. The camera was ~3 m from the distal end of the run pole. The camera is a Trailwatcher® motion-detection camera set to take photographs whenever an animal moves in front of the camera and with the flash on both day and night. The right panel shows a wolverine photographed at C&H station #31 by a Trailwatcher camera showing the ventral pattern used to identify the wolverine in the photograph.

481 List of Tables

482 Table 1. Posterior summary statistics from the spatial capture–recapture models fitted to the 2008
483 wolverine camera-trapping data from the mainland of Southeast Alaska near Petersburg. The 4
484 models considered were (1) a continuous, rectangular state-space with no behavioral response;
485 (2) a discrete approximation to the state-space using an 8-km grid; (3) a discrete state-space
486 using a 2-km grid with no behavioral response; and (4) a behavioral response added to the
487 second model; D is density per 1,000 km². Units of sigma are km.

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2 Figure 1

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1 Table 1

Model	Parameter	Mean	SD	2.50%	50%	97.50%
Continuous	D	5.799	1.252	3.627	5.714	8.496
	λ_0	0.058	0.01	0.041	0.057	0.079
	σ	6.393	0.556	5.415	6.349	7.599
	b1	0.013	0.002	0.009	0.012	0.017
	N	116.72	25.194	73	115	171
Discrete 8-km	D	8.195	1.592	5.474	8.061	11.644
	λ_0	0.045	0.007	0.033	0.045	0.061
	σ	6.812	0.472	5.982	6.774	7.845
	b1	0.011	0.001	0.008	0.011	0.014
	N	82.343	16.001	55	81	117
Discrete 2-km	D	8.591	1.689	5.677	8.414	12.267
	λ_0	0.048	0.008	0.034	0.048	0.065
	σ	6.322	0.489	5.426	6.298	7.342
	b1	1.273	0.197	0.928	1.261	1.698
	N	84.737	16.66	56	83	121
Discrete 8-km & behavioral effect	D	9.6688	2.3255	5.872	9.355	15.028
	λ_0	0.0087	0.0022	0.005	0.008	0.0137
	σ	9.8356	1.335	7.484	9.743	12.747
	b1	0.0055	0.0015	0.003	0.005	0.0089
	b2	2.5988	0.2652	2.088	2.595	3.1326
	N	97.152	23.367	59	94	151

Appendix B. A Master thesis on population structure and demographic history of wolverines in North America with an emphasis on peripheral populations (Frances 2008).

Introduction

Wildlife populations are structured by their surrounding geographical matrix so spatial information becomes an important component to consider when analyzing genetic patterns. The fusion of population genetics and spatial data allows innovative approaches to solving some of the challenging questions related to evolutionary processes occurring at different scales within wild populations. For example, spatial genetics (or phylogeography) have been useful in disentangling factors that produced observed patterns of genetic diversity across various geographic scales of a species' range (Avice 2004; Miller et al. 2006). Such understanding of the nonrandom distribution of genetic variation among individuals (McCauley 1992) can be crucial when developing management plans, especially when these plans are focused on species of conservation concern (Palsboll et al. 2007).

The wolverine (*Gulo gulo*) is a medium sized, highly vagile, holarctic carnivore (Family Mustelidae). In the last past 150 years, the wolverine's worldwide range has been greatly reduced (as much as 37%) by anthropogenic factors such as habitat destruction and hunting (Schreiber et al. 1989). Similar reductions in range have been documented in Scandinavia (Landa et al. 2000). The species is currently found in the boreal forests of Norway, Sweden, Finland, Mongolia, China, Russia, Canada, Alaska, and in a few northern states of the contiguous United States (Schreiber et al. 1989). Low population densities (Banci 1994), low reproductive potential (Weaver et al. 1996) and high sensitivity to human disturbance (Banci 1994) all contribute to the challenge of managing and conserving this species (Ruggiero et al. 1994). In the contiguous United

States, the wolverine has been petitioned twice for listing under the federal Endangered Species Act; both attempts have been declined by the USFWS (USFWS 2003). Wolverines are listed as “vulnerable” by the IUCN (Taylor 2000).

Terrestrial mammals like wolverine generally have larger core populations (found near the center of their distribution) that occur in suitable continuous habitats. Core populations tend to have higher neutral genetic variation than peripheral populations which are usually disconnected, smaller, and subject to different evolutionary forces (Allendorf and Phelps 1981; Lesica and Allendorf 1995; Mayr 1970; Safriel et al. 1994). Peripheral populations tend to be smaller, strongly affected by genetic drift, and may be influenced by lack of gene flow between core and peripheral populations (Hartl and Clark 1997). Two wolverine populations on the periphery of this species primary distribution in the Pacific Northwest are examined in this study.

One of these potentially isolated populations occurs in Southeast Alaska (Figure 1a), a region that is largely isolated from the remainder of continental North America (British Columbia and Yukon Territory) by glaciated Coastal Mountains. This mountain range is transected by a few large rivers (e.g. Stikine and Taku rivers) that may provide critical corridors for faunal exchange between interior and coastal populations (MacDonald and Cook 1996). Evidence of recent colonization down these natural corridors includes *Alces alces* (Hundertmark et al. 2003) and possibly *Martes pennanti* and *Puma concolor* (MacDonald and Cook 1996). A series of studies of mammals (Cook et al. 2001; Cook et al. 2006; Cook and MacDonald 2001; Dawson et al. 2007; MacDonald and Cook 1996; MacDonald and Cook 2007) recognize this region as having high levels of endemism (about 20% of known mammal taxa in the region are endemics)

including subspecies of other mustelids (Fleming and Cook 2002) and larger carnivores (Heaton et al. 2003; Heaton et al. 1996; Peacock et al. 2007; Talbot and Shields 1996; Weckworth et al. 2005). This region was repeatedly covered by glaciers during the Pleistocene epoch (Carrara et al. 2007; Mann and Hamilton 1995), but multiple refugia have been hypothesized along the outer coast of this region (Heaton et al. 1996; Carrara et al. 2007). Hence, glacial dynamics shaped species evolution and community assemblage in Southeast Alaska (Conroy et al. 1999; Heaton et al. 1996; Klein 1965)

A second potentially isolated population is located on the Kenai Peninsula (2300 km²; see Figure 1b) in southcentral Alaska and is connected to the mainland by a narrow isthmus (14 km wide) that may act as a barrier for animal dispersal. Hence, the wildlife of this region may be peripheral to connected continental populations. Numerous mammalian subspecies were described as endemic to this peninsula including *Martes americana kenaiensis* (Hagmeier 1958), *Gulo gulo katschemakensis* (Matschie 1918), *Canis lupus alces* (McCarley 1962), *Ursus arctos gyas* (Merriam 1918), and *Ursus americanus perniger* (Allen 1910). The distinctiveness of some of these nominal taxa was subsequently rejected by molecular studies (Paquet and Carbyn 2003; Tomasik and Cook 2005; Waits et al. 1998; Wooding and Ward 1997). During the last glacial maximum (LGM) this region was covered by glaciers (Miller 1958).

Both Southeast Alaska and Kenai Peninsula, along with the rest of North America, were affected by glacial dynamics during the Pleistocene (Carrara et al. 2007). On the continental scale, full glacial advances separated North America into at least 2 major unglaciated areas that may have supported refugial populations of mammals: the lower latitude region south of the Laurentide Ice Sheet in the contiguous United States

and the high latitude Beringia, a region that joined North America and Eurasia through the Bering Land Bridge (Hopkins 1967). As glaciers receded about 10,000 ybp at the end of the LGM, refugial populations began to recolonize previously glaciated regions (Hewitt 1996, 2001). These events had important consequences for the genetic structure of animal and plant populations (Hewitt 2004).

Three generalized hypotheses of wildlife recolonization in North America have been proposed: a) Single Source Recolonization: For a particular species, only animals north (or south) of the glaciers survived during the LGM and after deglaciation, these animals expanded southward (or northward) through a series of dispersal events that created a “leading effect” genetic signature (Miller et al. 2006). b) Dual Source Recolonization: populations of a particular species persisted both north (Abbott and Brochmann 2003; Hulten 1937; Pielou 1991) and south (Waltari et al. 2007) of the glaciers during the LGM. After the last glacial retreat, independently evolved northern and southern lineages came into secondary contact in mid-latitudes thus creating admixed populations or a distinct North/South discontinuity. c) Multiple Source Recolonization: wolverine populations survived in multiple refugia in North America (e.g. Beringia, High Canadian Arctic, Eastern, and Western Coastal Refugia) during the LGM. As glaciers retreated, wolverine recolonized previously glaciated areas thus creating admixture or multiple overlapping genetic signals in mid-latitude populations. The genetic structure of populations is determined by both common ancestry and ongoing gene flow. In vagile animals, high levels of gene flow should eventually distort the imprint of historic glacial events.

Due to high vagility, *G. gulo* is a good model for assessing spatial genetic structure and demographic history across western North America. The specific goals of this paper are: 1) To determine the geographic scale of genetic structure of North American wolverine populations. 2) To study the demographic history of these populations using coalescent approaches in light of the effects of the Pleistocene climatic variation by testing three primary hypotheses. 3) To explore genetic variability and divergence of wolverine populations in two peripheral regions: Southeast Alaska and the Kenai Peninsula through comparison with nearby continental populations. 4) To assess genetic distinctions of two New World subspecies, *G. g. luteus* and *G. g. katschemakensis*. 5) To discuss conservation implications for North American wolverine populations based on assessment of genetic variation.

Materials and Methods

Marker: Genomic DNA was isolated from tissue samples obtained from trappers and museum collections using a modified NaCl extraction protocol (Fleming and Cook 2002). The hypervariable left domain of the maternally inherited mitochondrial control region was used due to its high mutation rate, that is appropriate for analyzing intra specific variation in mammals (Avice 1994; Waits et al. 1998). Given that female wolverines are more philopatric than males (Cegelski et al. 2006; Chappell et al. 2004; Kyle and Strobeck 2001; Tomasik and Cook 2005), reduced levels of homogenization are expected between divergent mtDNA ancestral lineages due to matrilinear transmission (Godbout et al. 2008). Primers L15626 and H16498 were used (Shields and Kocher 1991;

Taberlet et al. 1996) to produce sequences of approximately 369 bp for both strands. *mtDNA* sequences were aligned using Sequence Navigator (Applied Biosystems, Inc.) and compared to reference sequences (Tomasik and Cook 2005).

Sampling: Sequences from 449 individuals were used, with 435 of those samples originating from North American populations and 14 from Eurasia. Of the North American samples, 321 were previously reported as follows: 7 were from California (Schwartz et al. 2007), 168 from the southern Rocky Mountains and central Rocky Mountains (Cegelski et al. 2006), 103 from Alaska and Northern Rocky Mountains (Tomasik and Cook 2005), 43 from the northern Rocky Mountains (Wilson et al. 2000), and 110 samples were newly added from Alaska and Canada. Of the Eurasian samples, 8 were from Scandinavia (Walker et al. 2001), 5 from Russia (Tomasik and Cook 2005), and 1 from Mongolia (Schwartz et al. 2007). These sequences represent 18 North American (Fig 2) and 3 Eurasian sampling localities. For phylogeographic analyses, all 21 localities were used (Table 1).

Phylogeographic Analyses: For consistency across available sequences, phylogenetic analyses included representative 318 bp sequences of all 449 wolverines (Table 1). Each insertion and deletion was treated as a fifth state.

Haplotype relationships were reconstructed using an unrooted Neighbor-Joining tree generated in the software package PAUP* 4.0 (Swofford et al. 1996). The model of evolution that best fit the data using Modeltest (Posada and Crandall 2001) with the Akaike criterion (Akaike 1974) was GTR+I. Clade support was assessed with 1,000 bootstrap pseudoreplicates (Felsenstein 1985) and considered highly supported with a bootstrap value ≥ 70 (Hillis and Bull 1993). To examine the degree of connectedness

among all haplotypes, a Median Joining Network (MJN) was constructed using the program Network 4.2.0.1 (Fluxus Technology 2007). Haplotype networks are more sensitive than tree based approaches for assessing fine-scale structure within species (Bermingham and Moritz 1998; Posada and Crandall 2001; Smit et al. 2007). Population frequencies were included in the MJN to illustrate the occurrence of unique and rare haplotypes. For all other analyses, only North American wolverine localities with a sample size of 8 or larger were analyzed (i.e., all North American samples except California), thus increasing sequence length to 369 bp.

Spatial Genetic Structure: The program Alleles in Space (Miller 2005) was used to assess whether, on average, nonrandom patterns of genetic diversity (i.e., spatial genetic structure) exist over the studied landscape. Spatial autocorrelation analysis (Sokal and Oden 1978) allowed inference related to the spatial scale of putative genetic structure (Barbujani 2000; Manel et al. 2003). Autocorrelation (A_y) was quantified as the average genetic distance between pairs of individuals that fell into distance class y . Analyses were initially performed using 10, 20 and 30 distance classes and the unequal distances / equal number of observations option. A randomization procedure (5,000 replicates) was used to identify distance classes with average genetic distances significantly larger or smaller than random expectations. The extent of genetic structure revealed by this analysis is obtained by identifying the transition point that autocorrelation coefficients switch from values less than average to values that are greater than average (Clark and Richardson 2002). The Mantel Test (Mantel 1967; Sokal et al. 1995) was performed using the software package Arlequin (Excoffier et al. 2005) to assess Isolation by Distance (IBD). The Landscape Shape Interpolation procedure (Miller 2005) was used to facilitate

visualizations of patterns of *mtDNA* variation among individuals by creating a 3-dimensional surface plot where *x* and *y* axes correspond to cardinal directions (projected in Universal Transverse Mercator) and the *z*-axis represents residual genetic distances. This analysis produces a graphical representation of patterns of diversity across the sampled landscape with peaks in those areas with high genetic distances. To obtain an overview of the general pattern across most of the wolverine's North American distribution, a distance weight value of 0.15 was used.

To detect dissimilar patterns among populations, non-metric multidimensional scaling (MDS) was performed based on a genetic distance matrix (F_{ST} values, obtained in Arlequin 3.11; (Excoffier et al. 2005) between the different haplotypes. The stress value was also calculated. Low stress values (< 0.001) indicate that the data are clustered in well differentiated groups, while a high value indicates the opposite. To further illustrate the geographic structure of these wolverine populations, the SAMOVA 1.0 (<http://web.unife.it/progetti/genetica/Isabelle/samova.html>) algorithm (Dupanloup et al. 2002) was implemented. This approach identifies partitions of geographically adjacent sampling areas that are maximally differentiated based on sequence data. The number of North American population partitions was estimated by running 100 simulated annealing steps and examining maximum indicators of differentiation (F_{CT} values) with the program instructed to identify $K=2$ through $K=15$ partitions.

Molecular Diversity and Historical Population Dynamics: The software package Arlequin 3.11 (Excoffier and Schneider 2005) was used to calculate nucleotide diversity (π) and haplotype diversity (h). The probability that 2 randomly chosen homologous nucleotides are different is π (Excoffier et al. 2005) and varies from 0 (no divergence) to

over 0.10 for deep divergence (Grant and Bowen 1998), while h is the probability that 2 randomly chosen haplotypes differ (Excoffier et al. 2005) and varies from 0 to 1 (Grant and Bowen 1998). Private alleles (alleles only found in 1 locality) were assessed by looking at the haplotype network. A hierarchical analysis of molecular variance (AMOVA) was run to evaluate population genetic structure. Following the Pleistocene refugia hypotheses, 3 groups were identified based on whether a sampled population was located in a previously glaciated or deglaciated (refugia) area. One group included populations found in the former Beringia refugium (NWAK, NAK, CAK), a second group included populations located in southern refugia (RMF, GA, CB, WY, ID) and the last group included all populations that are located on previously glaciated locations.

To test the difference between core and peripheral populations, another AMOVA was performed with the following groupings: peripheral (KP, SEAK, both isolated from remaining populations by geographical features, and WY, ID, RMF, CB, GA, each now found in the southern edge of the wolverine distribution) and core (remaining 11 localities). A second order polynomial regression plot was obtained using latitude (decimal degrees) as the independent variable and nucleotide diversity as the response variable. Data were analyzed using Excel 2007 (Microsoft, Inc) to obtain regression equations and R^2 values. The demographic history of each the New World sampling localities was assessed using the Metropolis-Hastings algorithm as implemented in FLUCTUATE v1.4 (Kuhner et al. 1998). Ten short chains (sampling increments of 10; 1,000 steps per chain), 2 long chains (sampling increments of 10, 20,000 steps), and a random starting tree were used in each run. Convergence of results was assessed through 5 separate runs with different starting seed numbers. Positive values of g indicate a

demographic expansion while negative values indicate a population decline (Kuhner et al. 1998). Conservative confidence intervals of (+/- 3 SD) were used to test significance of difference from zero (Lessa et al. 2003). The neutrality tests of Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) were used as an additional, independent assessment of changes in population sizes. Significance of F_s and D were evaluated by using 10,000 random permutations in Arlequin 3.11 (Excoffier et al. 2005). For these 2 tests, significant negative values indicate deviations from neutrality and could indicate population expansion (Excoffier et al. 2005). For these last 2 demographic history analyses, populations were also lumped together in 3 groups following the same criteria as the AMOVA. Lastly, the raggedness index (Harpending 1994) from the mismatch distribution was calculated using the software package DnaSp (Version 4. 10. 9). The raggedness index (r) is directly related to the degree of mismatch between the observed and expected distributions. Values of r lower than 0.05 indicate population expansion (Excoffier et al. 1992). This analysis was only performed for the Beringia Refugium, Southern Refugium and Glaciated Areas groups.

Results

Phylogeographic Analyses: Control Region sequences yielded 318 bp after alignment and trimming of ambiguous ends and a total of 35 haplotypes and 22 parsimony informative sites found (Appendix 1 a, b). The unrooted NJ tree (Figure 3) showed a star phylogeny for most North American haplotypes. The only supported node (bootstrap value of 72) included 2 Eurasian haplotypes (Russia and Scandinavia). Two

Californian haplotypes (Hap 33 and Hap 34) were grouped with a haplotype from the Beringia refugium (Hap 33) but with low support (54). A single Ontario haplotype (Hap 22) is weakly related to that clade. For the MJN (Figure 4), all haplotypes were minimally diverged, separated by 1 to 3 mutational steps. A single Californian haplotype is shared with the Beringia Refugium. The next closest North American haplotype (haplotype 2) is found in several populations (Kenai Peninsula, Beringia Refugium, Russia, Nunavut, Southern Alaska and the purported southern refugia) and it is 3 mutational steps distinct. The Scandinavian haplotype (haplotype 35) was 3 mutations from haplotype 32 (found in Russia). Haplotype 1 showed the highest frequency ($n = 200$) and was found in all the North American localities except California. Private alleles were found in Southern Alaska, Southeast Alaska, and Ontario. Ontario had 4 unique haplotypes. This expanded study indicated that Kenai Peninsula had no private alleles (Tomasik and Cook 2005) and shared 2 haplotypes with Southern Alaska (hap 1 and 2). Southeast Alaska and Yukon Territories shared 3 haplotypes (hap 3, 13 and 17).

Spatial Genetic Structure: For population level analyses (369 bp), 34 haplotypes and 21 informative sites were found. The Mantel Test indicated that genetic distance was weakly correlated with geographic distance across population pairs (results not shown $R^2 = 0.198$, $P\text{-value} < 0.05$). Spatial Autocorrelation Analysis indicated low heterogeneity in the A_y values despite the large geographic area analyzed. A_y values did not change linearly with geographic distance (Figure 6), corroborating an overall absence of spatial pattern (Diniz-Filho and Telles 2002; Miller et al. 2006). Landscape Shape Interpolation (Figure 7) showed similar results regardless of grid size. A general pattern of increasing genetic distance in mid latitudes was observed. After the RE highest peak, genetic

distances decreased northward with some lower peaks in WL (British Columbia) and SAK. Highest genetic distance values were found in the mid-area of the sampling scheme. SAK, WL, RE, and CB all exhibit high genetic discontinuity that reflects a South/North orientation. Three East/West slopes were uncovered also, highlighting differences that occur in localities at the similar latitudes. The first notable difference is between GC and WL, but these 2 populations do not show a high F_{ST} value (0.008, see Table 2). This example illustrates the higher resolving power of this analysis, which reports genetic distance on an individual basis instead of a population scale. Landscape Shape Interpolation plots every sampled animal and shows inter-individual genetic distances without population assignment assumptions thus providing a more detailed picture of geographic partitioning of the genetic variation. The second difference is between 2 individuals from SAK and exemplifies the high levels of intra-population genetic diversity reported by the AMOVA. This population did not exhibit high nucleotide diversity (0.00479, see table 3). Finally, NWT/NUT populations show a small, yet still significant East-West cline that may indicate that at least 1 of these regions has an independent population history.

Global F_{ST} (calculated among all localities) was 0.297, with values ranging from 0 to 0.607 (Table 2). The MDS plot (Figure 8) (obtained from the F_{ST} values) displays weak genetic structure (stress = 0.148) in the multi-dimensional space (Cox and Cox 2001). Geographically proximate populations such as NUT and NWT appear distant in the MDS plot, while geographically distant populations such as YT and WY are proximate, a finding that illustrates the overall lack of regional genetic structure. Nonetheless, a few populations appear to be isolated from the others including: CB,

NUT, SEAK and KP. Russia appeared isolated from the rest of the sampling localities. SAMOVA (Figure 9) did not identify genetically defined sampling localities. The F_{CT} value increased as the K value increased, again reflecting an overall lack of geographic structure. For the highest F_{CT} value ($K=15$), every population was placed in its own group except that GC and WL, WY and GA, and ID and RMF were paired together.

Molecular Diversity and Historical Population Dynamics: Haplotype diversity (h) ranged from 0 to 0.011537 and nucleotide diversity (π) ranged from 0 to 0.8571 (Table 3). Highest values for both π and h were ONT. A second order polynomial ($R^2 = 0.231$, Figure 10) best fit the data, indicating that localities in mid latitudes of the sampling scheme had highest values of π . ONT represents an outlier given that it has a value of π that is an order of magnitude larger than other localities (if excluded, the R^2 value increases to 0.463). SAK showed the highest number of private alleles (6), followed by ONT (3), YT, SEAK, WL (2 each) and RE, and NWT (1 each). The AMOVA showed (Table 4) that 1.89% of the genetic variance was found among the 3 groups, 27.65% between populations within groups (global $F_{ST} = 0.277$) and the remaining 70.47% within populations. The 3 groups were extremely similar to each other regardless of their hypothesized Pleistocene history. For the core vs peripheral AMOVA, only 0.11 % of the variation was found between these 2 groups. The population growth parameter (g) indicated that 8 localities (CAK, NWT, RM, SAK, UT, WL, WY, and YT; Table 5) displayed signals of population expansion (estimates of exponential growth larger than zero). Neither Tajima's D nor Fu's F_s showed significant negative values for those populations, which indicates that this marker is evolving in a neutral fashion (Excoffier et al. 2005; Fu 1997; Tajima 1989). When the populations were sorted into 3 groups, the

southern refugia showed a mixed pattern of population expansion and stability ($D = -0.06151$, $F_s = 0.642$) and the glaciated areas showed clear signs of population expansion ($D = -1.06824$, $F_s = -12.656$), while the Beringia refugium showed apparent signs of population stability ($D = 1.05215$, $F_s = 0.036$). These analyses were not significant, but Tajima's D , is known to be conservative, and non-significant values may identify circumstances of population expansion (Lessa et al. 2003). Mismatch distribution and raggedness indices showed that populations in the Beringia refugium reflect stability ($r = 0.0644$) while the southern ($r = 0.0402$) and glaciated populations showed signs of population expansion ($r = 0.0486$).

Discussion

This study of mtDNA variation corroborates previous findings indicating that female genetic variation is spatially partitioned in wolverines (Cegelski et al. 2006; Schwartz et al. 2007; Tomasik and Cook 2005). Although wolverines are capable of dispersing long distances, females are known to be philopatric, with home ranges significantly smaller than those of males (Hornocker and Hash 1981; Magoun and Copeland 1998). Juvenile males show the highest vagility while establishing their own territories, further exemplifying the sexual dispersal bias of this holarctic carnivore (Banci and Harestad 1988; Magoun and Copeland 1998; Persson et al. 2003).

Spatial Structure: The Mantel test showed a weak IBD; even when peripheral populations were discarded. Spatial Autocorrelation Analysis did not show a clear pattern, because A_y values did not change linearly with geographic distance. Therefore, presence of barriers to gene flow seems to have a stronger effect on spatial genetic

variation than geographic distance. AMOVA failed to depict any differences between the 3 proposed groups (1.89 %). Differences among populations within groups was considerably higher (27.65 %), especially for an extremely vagile carnivore, and likely reflects the relatively low levels of gene flow found in female wolverines in the recent past.

The SAMOVA analysis failed to clearly identify population groups, the F_{ct} value constantly increased as k got larger. Schwartz et al (2007) found 11 distinctive groups when they included Old World and Californian samples. Those two populations were not included in the population-level analyses due to their small sample size and short sequence lengths, but they are relatively distinctive from other North American samples (Fig. 3 and 4). Schwartz et al (2007) also reported that localities found in the southern Rocky Mountains grouped together (also supported by this study for $k = 13$). Given that these localities are similar, they all might have had originated from the same source populations. The MDS plot also showed poor sorting power (low stress value) when placing localities into distinct groups. Nonetheless, a few populations were distinct including Russia, perhaps reflecting the independent evolutionary history of the Old World *G. gulo* after the LGM (see taxonomy section). The CB population also appeared to be distinct (also seen in the genetic landscape shape), corroborating the findings of Cegelski et al (2006) and Schwartz et al (2007). Those studies reported that southern and northern Rocky Mountains populations were different (based on both nuclear and *mtDNA* data). This distinction was demonstrated in the MDS plot but lack of a significant phylogeographic break between glaciated and southern refugia populations suggests that these 2 areas share similar population histories.

The pattern of evolutionary relationships among haplotypes (Fig. 3) indicates that a few are distinctive while most are part of a large star phylogeny. Wolverine populations on deglaciated sites share haplotypes and common haplotypes are broadly distributed across the landscape, with geographically distant populations often sharing haplotypes. Similar patterns of minimal molecular variation among recently expanded populations at mid-latitudes in central Canada are common within major phylogeographic groups that have been examined in North America (Demboski et al. 1999; Hewitt 2004).

Three East-West clines (Fig. 7) detected are located along the northwestern edge of the North American continent and these may indicate that distinct eastern refugia, not previously considered, could have played an important role in shaping overall genetic structure of *G. gulo* (e.g. High Canadian Arctic or Appalachian refugia).

Historical population dynamics and hypotheses testing: Most alleles are roughly similarly divergent from each other; a pattern that may reflect a recent population expansion and/or deviation from neutrality (e.g. recent selective sweep, selection against slightly deleterious alleles) for these wolverine populations. To distinguish between population expansion and selective sweeps, multiple unlinked markers should be considered in future studies. If these also show signs of deviations from neutrality, then we can rule out the occurrence of a selective sweep (because selection acts on a locus by locus basis) and conclude that a population expansion took place (Lessa et al. 2003). Furthermore, Ramos-Onsins and Rozas (2002) showed that both mismatch distributions and raggedness indices are conservative measures.

Highest nucleotide diversity values were found at mid latitudes (Fig 9). Multiple refugial populations that survived the LGM independently recolonized the previously

glaciated areas leading to genetic signatures that overlap in these mid-latitude populations. Miller et al (2006) found a similar pattern for the red tree vole (*Phenacomys longicaudus*) and concluded that this species recolonized from both the north and south. Nonetheless, they also found 2 very divergent clades, 1 southern and 1 northern. Here, no distinct clades were identified (see Figure 3 and 4). Low nucleotide and haplotype diversity values (Table 3) and absence of private alleles in the southern Rocky Mountains are not signature features of refugial populations. The 4 haplotypes found in Rocky Mountain populations (1, 2, 8, 11) likely originated elsewhere and expanded south of the glaciated areas. These data reject the hypothesis that southern Rocky Mountains served as source populations for high latitude recolonization by wolverine.

Three populations showed potentially reflecting the influence of refugial origin other than Beringia: California, Nunavut, and Ontario. Californian populations were genetically more similar to Eurasian samples than to the other North American samples (Schwartz et al. 2007). Because more than 20 major glaciations events have now been identified during the Pleistocene, recolonization of glaciated areas may have occurred multiple times. Wolverines may have colonized California during an interstadial prior to the LGM and then survived in an isolated refugial south of the ice sheets. Haplotype 33 is shared between California and Beringia and may reflect incomplete lineage sorting because not enough time was given for these 2 areas to be reciprocally monophyletic. An earlier colonization of North America in an independent southern refugia has been reported for another highly vagile carnivore, the Mexican grey wolf (Leonard et al. 2005). When the glaciers receded, Californian wolverine populations apparently remained isolated as there is no effective genetic signal (no shared alleles) that they

contributed to recolonization elsewhere in North America, but Washington and Oregon populations need to be surveyed. Another possible explanation for this observed pattern is homoplasy. To test this possibility, Schwartz et al (2007) also studied nuclear markers and found that these samples were not related to Rocky Mountains populations. These results further support the independent evolutionary history of the Californian samples and are not consistent with the Single Source Recolonization hypothesis.

Nunavut wolverine populations are genetically distinctive from other localities (Fig 8). NUT and NWT have a unique, highly frequent allele (Haplotype 9) that may reflect the independent evolutionary history of these populations (Fig. 4). Nonetheless, these populations (along with the NWT) showed clear signs of expansion, perhaps indicating that wolverine populations persisted elsewhere north of the glaciers in high arctic refugia in eastern Canada (Rand 1954). Wolverine later expanded into Nunavut and Northwest Territories following the LGM, but further geographic sampling and especially independent loci are needed to corroborate this hypothesis.

For eastern Canada and northeastern USA, palaeobotanical evidence supports the presence of an isolated unglaciated area in the Appalachian Mountains (Pielou 1991). Species of eastern white pine and eastern hemlock survived the Wisconsin glaciation in refugia in the eastern foothills of the Appalachians and on the adjacent coastal plains (Pielou 1991). Previous studies have also identified refugial populations for the short-tailed shrew (Brant and Orti 2003) and spotted salamander (Zamudio and Savage 2003) in the Appalachians as well as a distinct eastern clade for the black bear (Wooding and Ward 1997). Wolverine appear to be tightly associated with alpine vegetation, cold temperatures, and spring snow cover (Aubry et al. 2007) which could have been present

in a eastern refugium. Our sampling assessment did not survey eastern North American populations of wolverine and precludes analyses of potential contributions to northward expansion from an eastern refugia (Pielou 1991). Four private alleles were found in the most eastern population (ONT) these were not closely related to each other (Fig. 4) perhaps reflecting admixture from multiple refugial sources. Few samples representing the Ontario locality combined with the need to sample eastern populations such as Quebec, and Labrador preclude testing the eastern refugial hypothesis.

Overall events that contributed to mitochondrial diversity in mid-latitude populations appears to be historical population expansion primarily from the Beringia refugium, followed by contemporary low levels of female gene flow as reflected by relatively high between populations F_{st} values. Range expansion has significant consequences for shaping the genetic structure of high latitude species (Nichols and Hewitt 1994). Once glaciers fully retreated, and the region had been colonized by wolverine, inter-population migration of female wolverine may have decreased leading to higher between population differentiation (Table 2).

Multiple fossil records south of the glaciers during the LGM (Bryant 1987) suggest that modern North American wolverine populations might have descended from these southern refugial populations. However, mtDNA sequences reject the Southern Source Recolonization hypothesis and indicate that refugial populations found in Beringia gave rise to most contemporary North American wolverine populations (except California) after the last glacial maximum. Previous studies (Cegelski et al. 2006; Tomasik and Cook 2005) identified Beringia as the likely origin for extant wolverine populations in North America. A similar pattern of a post-Pleistocene Beringian origin

has been suggested for tundra voles (Galbreath and Cook 2004), moose (Hundertmark et al. 2003; Hundertmark et al. 2006), ermine (Fleming and Cook 2002) northern red-backed vole (Cook et al. 2004), and arctic ground squirrel (Eddingsaas et al. 2004). A southern coastal refugial origin has been proposed for cinereus shrew (Demboski and Cook 2003), Pacific marten (Small et al. 2003; Stone and Cook 2002), and wolf (Weckworth et al. 2005). Californian wolverines may have been part of an earlier colonization that persisted in this southern coastal refugium., but this population did not contribute to the gene pool of the remaining North American populations.

Taxonomy: *Gulo* is descended from *Plesiogulo* (Kurten and Anderson 1980) and is the sister taxon of the genus *Martes* (e.g., fisher; (Dragoo and Honeycutt 1997). There are Pleistocene fossil records of wolverine in both Eurasia and North America (Hibbard et al. 1965), but New World fossils appear to be older (Kurten and Anderson 1980). The taxonomic status of extant Palearctic and Nearctic wolverines has been controversial. The current taxonomic view is that *Gulo gulo* is the only extant representative of the genus (*Gulo schlosseri* and *Gulo gidleyi* are extinct forms; (Hall 1936; Kormos 1914) and that there are 2 subspecies: *G. gulo luscus* in North America, and *G. g. gulo* in Eurasia (Bryant 1987; Krott 1960; Kurtén and Rausch 1959; Wilson and Reeder 1993). *G. g. vancouverensis* on Vancouver Island (Goldman 1935), *G. g. katschemakensis* on the Kenai Peninsula (Matschie 1918), and *G. g. luteus* in California (Elliot 1903) have also been described as putative subspecies based on morphological data.

Results are consistent with previous findings (Schwartz et al. 2007; Tomasik and Cook 2005) that report incomplete lineage sorting between New and Old World wolverines, although further sampling is needed. The star phylogeny (Fig 3) and overall

low sequence divergence support the hypothesis that North American and Eurasian wolverines are a single evolutionary lineage or species, but sampling remains limited especially in eastern Asia. A major phylogeographic discontinuity in southeastern Siberia has been reported in other mammals (e.g. *Myopus schisticolor*; Fedorov et al. 2008), further illustrating the need for more extensive sampling of wolverine in Asia to elucidate their biogeographic history.

Multiple shared haplotypes between the Kenai Peninsula and inland Alaska and lack of a distinct clade for this region suggest that *G. g. katschemakensis* may not represent a distinct evolutionary unit (Tomasik and Cook 2005). Finally, Schwartz et al (2007) reported the independent evolutionary history of the California samples from the Rocky Mountain samples. The shared allele (haplotype 33) between California and Beringia may also reflect incomplete lineage sorting of ancestral alleles. The status of the Californian subspecies *G. g. luteus* as a distinct trinomial remains unclear.

Peripheral Populations and Conservation Implications: Core and peripheral populations differed minimally (Table 4) indicating that peripheral populations do not show less genetic variation than core populations as would be expected by theory (Allendorf and Phelps 1981; Lesica and Allendorf 1995; Mayr 1970; Safriel et al. 1994). Kenai Peninsula is not highly distinctive (Fig. 8). The wolverine of the Kenai Peninsula did not show any private alleles.

Southeast Alaska in contrast has 2 private alleles, indicating the possibility of reduced gene flow with neighbor Canadian populations. There is limited female gene exchange at the local scale between these populations and their correspondent inland populations (F_{st} of 0.2159 for SEAK/YT and 0.294 for SEAK/WL), but these are not

unusually low levels of gene flow when compared to the other F_{st} values (Table 2). Wolverine in these areas do not represent a coastal refugial population in the Alexander Archipelago (Carrara et al. 2007). Furthermore, Southeast Alaska wolverine showed 2 private alleles, a potential indication that this region is partially isolated from nearby populations. Within southeast Alaska, there were no significant differences between island and mainland samples, but wolverine only occur on nearshore islands (Mitkof, Kupreanof and Kuiu). Tomasik and Cook (2005) recognize Southeast Alaska as a distinctive population. Our expanded analysis (increasing sample size in Southeast Alaska from 12 to 31) and new samples from nearby populations (YT, $n = 23$, and WL, $n = 24$) corroborates their preliminary findings. This result for wolverine contrasts with previous studies of another coastal mustelid that identified the potential signature of a Pacific coastal refugium (Carrara et al. 2007) in Southeast Alaska ermine (Fleming and Cook 2002).

High F_{st} values indicate that the females are not successfully migrating among populations but others (Cegelski et al. 2006; Chappell et al. 2004; Kyle and Strobeck 2001; Tomasik and Cook 2005) report that male wolverines appear to be the primary dispersers (especially subadult males). Even though male biased migration could prevent inbreeding depression in these areas, the lower number of incoming females (represented by high F_{st} values) might have dramatic implications from an ecological standpoint. Moreover, Krebs et al. (2004) demonstrated that in harvested areas, males (especially subadults) had lower survival than females. If the predominant dispersers exhibit decreased survival and females are not successfully migrating through the landscape, intensively harvested populations might be of special conservation concern. Furbearer

sealing records by the Alaska Department of Fish and Game for the peripheral populations of Southeast Alaska (GMU 1-5) and Kenai Peninsula (GMU 7 and 15) indicate an average annual harvest of 24 and 14 animals respectively over the last 10 years (Alaska Department of Fish and Game 2004). Wolverine populations are believed to be able to sustain a harvest rate of 6 % of the population per year (Krebs et al. 2004). In these peripheral regions, wolverine populations might decline without the constant inflow of females (Krebs et al. 2004), so interior British Columbia wolverines (those east of the Coast Range) may be important to maintain the viability of Southeast Alaska (Lofroth and Ott 2007). Changing climates may also impact these mesocarnivores. (Magoun 1985) reported that den abandonment was probably forced by snow melt and Aubry et al (2007) found a close correlation between wolverine presence and spring snow cover. Global climate change is heavily impacting higher latitudes with consistent indications of spring warming of arctic terrestrial regions and earlier disappearance of snow cover (IPCC 2007). Lack of spring snow cover represents an additional external pressure to the already harvested wolverine populations.

Conclusion

For the North American wolverine, there is an overall lack of large scale geographic structure (weak IBD), but moderate to high F_{st} values between adjoining populations. A pattern of Holocene population expansion into previously glaciated regions along with limited ongoing levels of gene flow may explain these findings. In

general, for northern localities show more genetic diversity than southern localities, with relatively high levels of nucleotide diversity found in mid latitudes populations.

Beringia may have been a primary source for the recolonization of most of western North America with the possible exception of California and the most eastern populations (Ontario and Nunavut) considered in this study. The previously glaciated Canada and eastern Alaska show signs of recent population expansion while southern populations do not harbor refugial signatures and appear to be the result of a recent population expansion from northern areas.

Californian wolverines are distinct from most North American populations but share one haplotype with Beringia and Russia, a finding indicating that wolverines might have colonized from Beringia prior to the LGM. When glaciers retreated, Californian wolverine apparently remained isolated and did not contribute to the genetic diversity of the remaining contiguous North American wolverine populations. Nunavut and NWT are genetically distinctive but appear to be the result of a recent population expansion, perhaps reflecting the influence of a separate High Canadian Arctic refugium. Ontario was also genetically distinctive (e.g. high nucleotide diversity); this diversity could reflect admixture from eastern (e.g. Appalachian) refugial populations. Lack of eastern samples makes this interpretation inconclusive.

Southeast Alaska wolverine showed no signature of a history of persistence in Pacific Coastal Refugia. In spite of the relatively recent wolverine invasion of the coast, females show low levels of exchange with nearby areas. Kenai Peninsula does not seem to represent a distinctive population.

Fossil records from the Pleistocene indicate that multiple mammals followed the recolonization of their preferred vegetation type closely following the LGM (Hibbard et al. 1965). This study adds to the emerging pattern found among North American vertebrate populations (e.g. (Arbogast 1999; Brant and Orti 2003; Byun et al. 1997; Conroy and Cook 2000; Klicka and Zink 1997) and specifically, boreal carnivores (e.g., fisher, Wiseley et al. 2004; black bears, Peacock et al. 2007; brown bears, Barnes et al. 2002; wolves, Weckworth et al. 2005; and lynx, Schwartz et al. 2003 that landscape features and geological history affect these species' spatial genetic structure. Although carnivore species show similar life history traits, they do not necessarily exhibit the same biogeographic histories.

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Figures

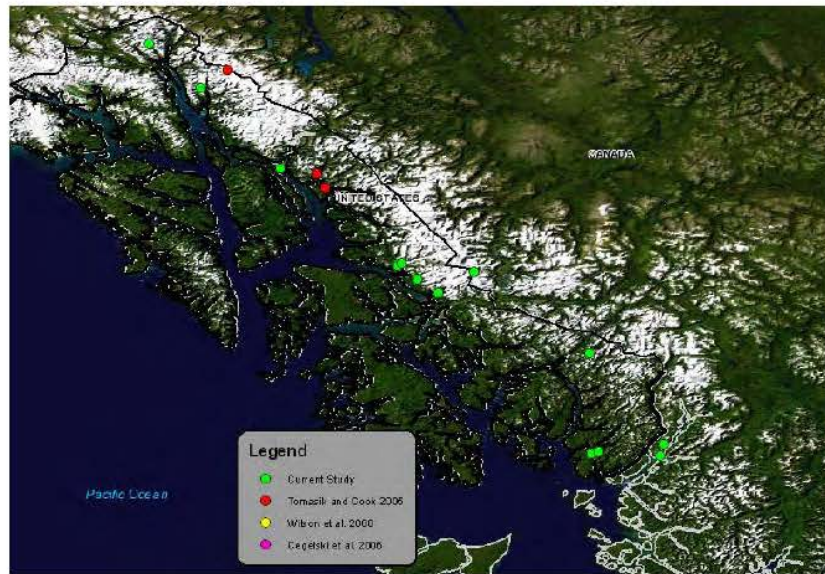


Figure 1a: Close up of Southeast Alaska

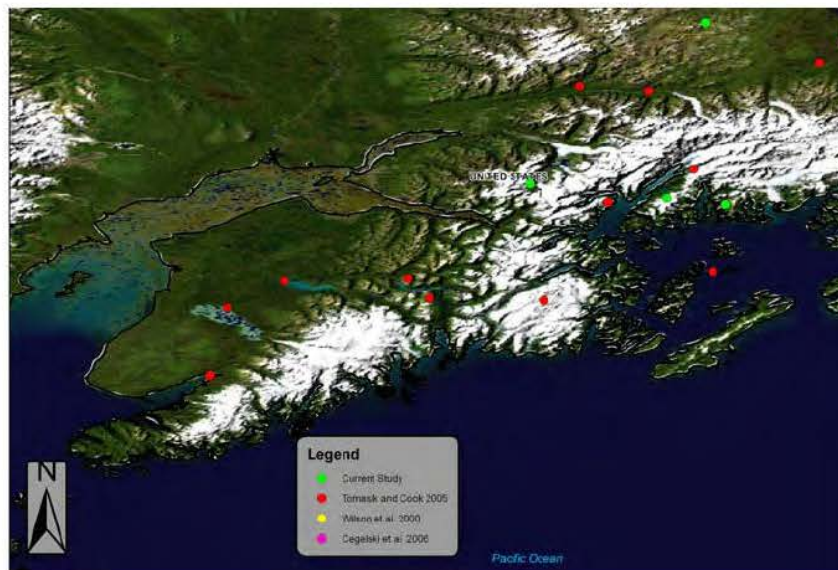


Figure 1b: Close-up of the Kenai Peninsula.

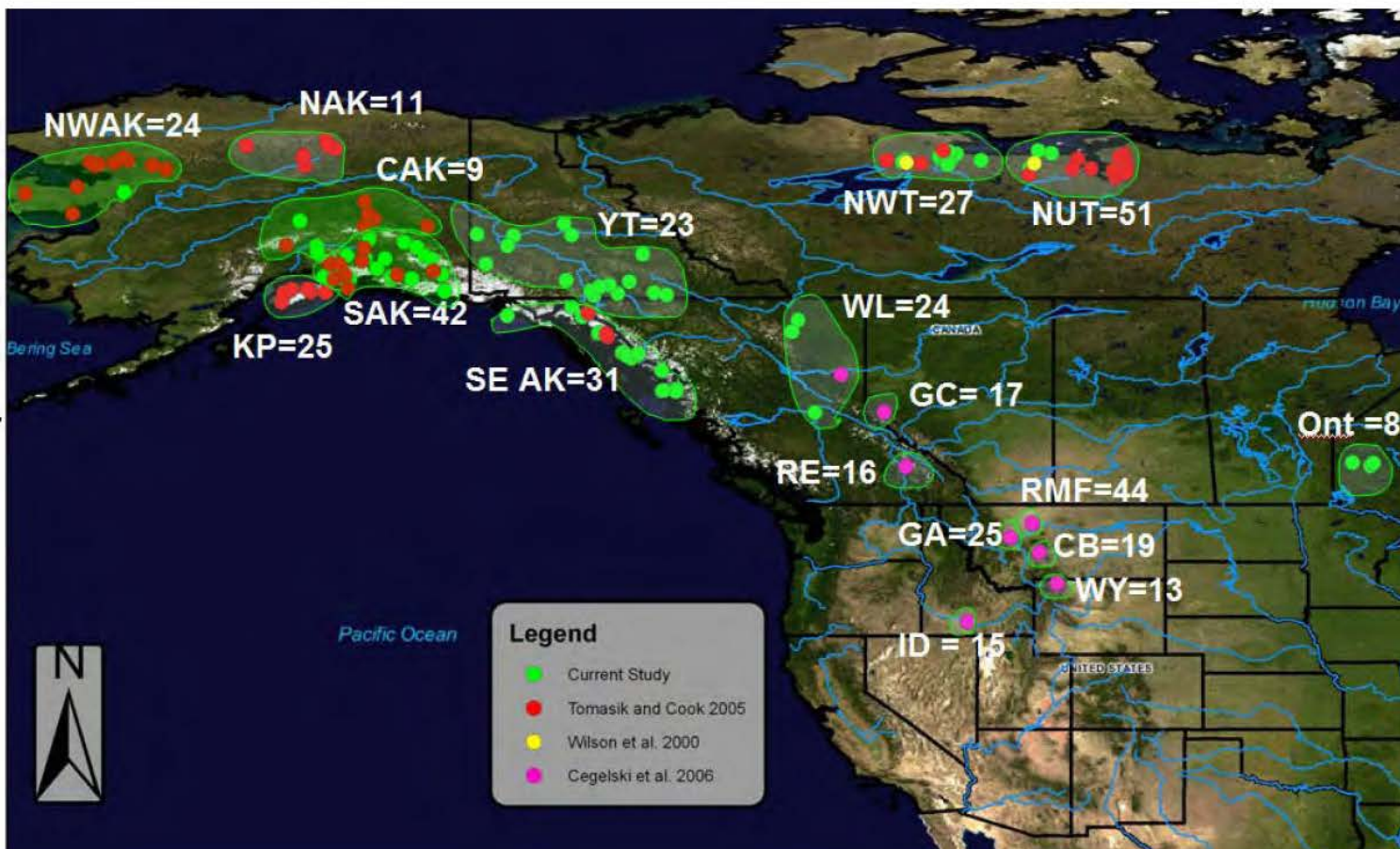


Figure 2: Map with all the North American sampling localities. Light blue dots represent samples taken from Cegelski et al 2006, orange (present study), green (Wilson et al 2000) and red (Tomasik and Cook, 2005). The abbreviations mean: WAK (Western Alaska), NAK (Northern Alaska), CAK (Central Alaska), SAK (Southern Alaska), KP (Kenai Peninsula), SE AK (Southeast Alaska), NWT (Northwest territories), NUT (Nunavut), Ont (Ontario), WL (British Columbia), GC (Alberta), RE (Revelstoke), RMF (Montana 1), GA (Montana2), CB (Montana 3), ID (Idaho), WY (Wyoming). Sample sizes are indicated in white numbers.

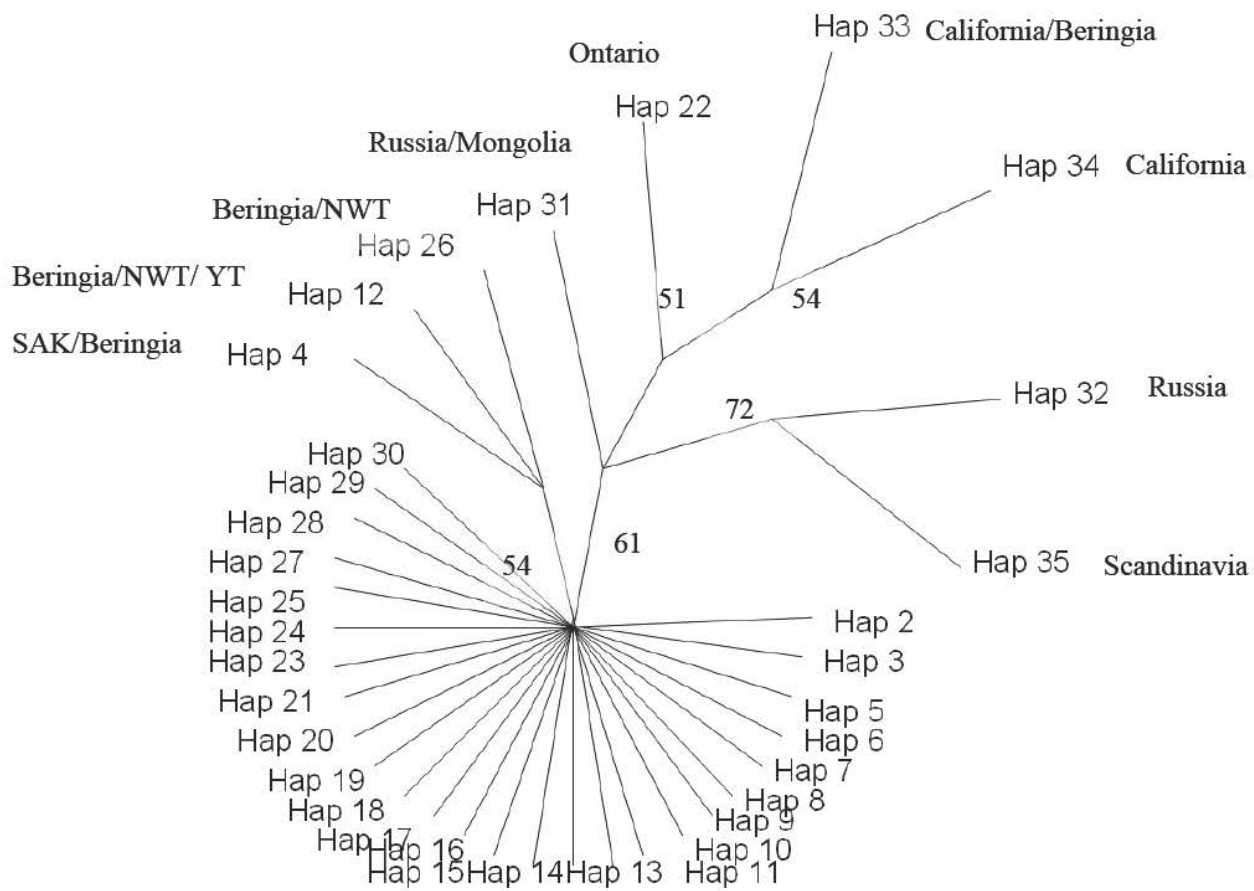


Figure 3: Neighbor Joining Tree (NJ) based on GTR+I distances displaying all 35 *Gulo gulo* haplotypes found. Internal node numbers indicated Bootstrap support based on 1,000 iterations.

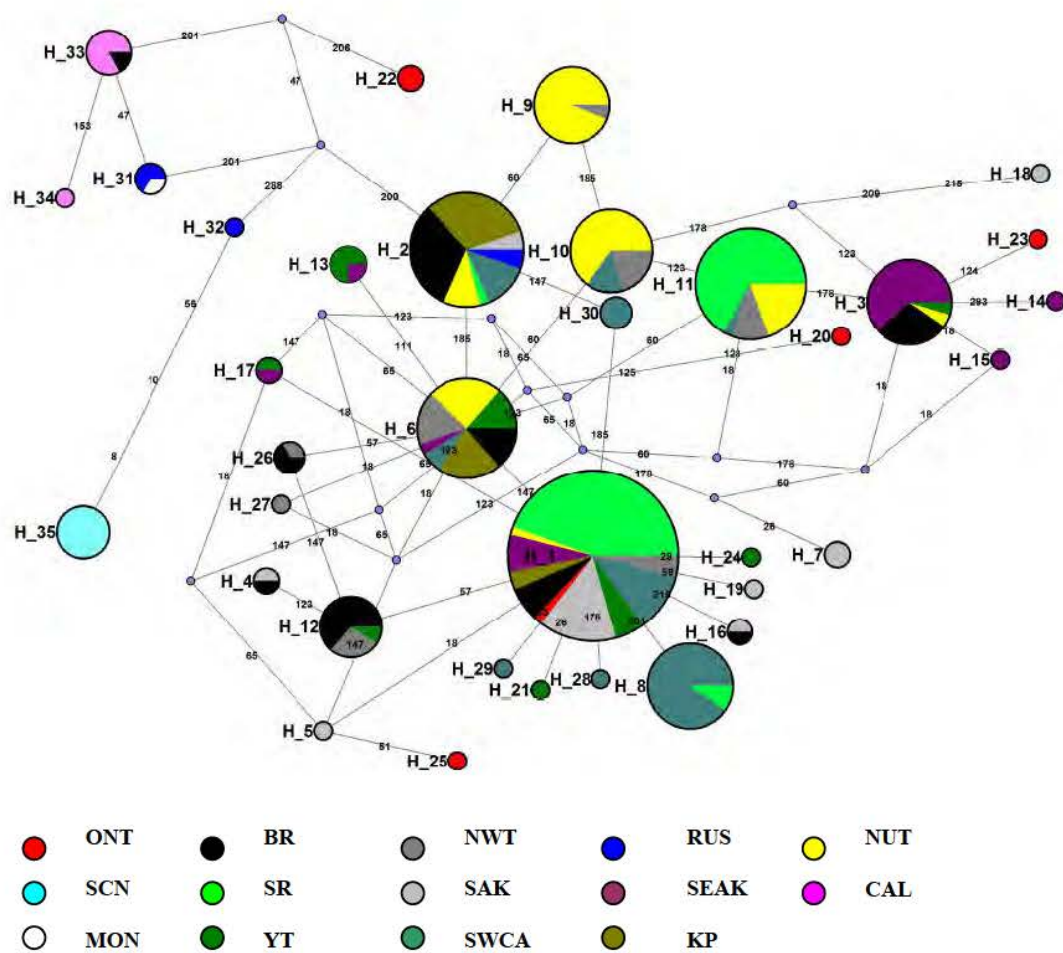


Figure 4 Median Joining network (MJN) illustrating the 35 *Gulo gulo* Control Region haplotypes (318 bp) in this study. See legend for correlation between haplotype labels and populations. Pie diagrams indicate frequencies of populations within haplotypes. Black numbers indicate mutational steps between 2 given haplotypes. Light blue circles represent median joining vectors. The abbreviations mean the same as in Figure 1 but for: SCN (Scandinavia), MON (Mongolia), BR (Beringia Refugia): NWAK, NAK, CAK, SR (Southern Refugia): RMF, GA, CB, WY, ID, CAL (California), SWCA (Southwestern Canada): WL, GC, RE.

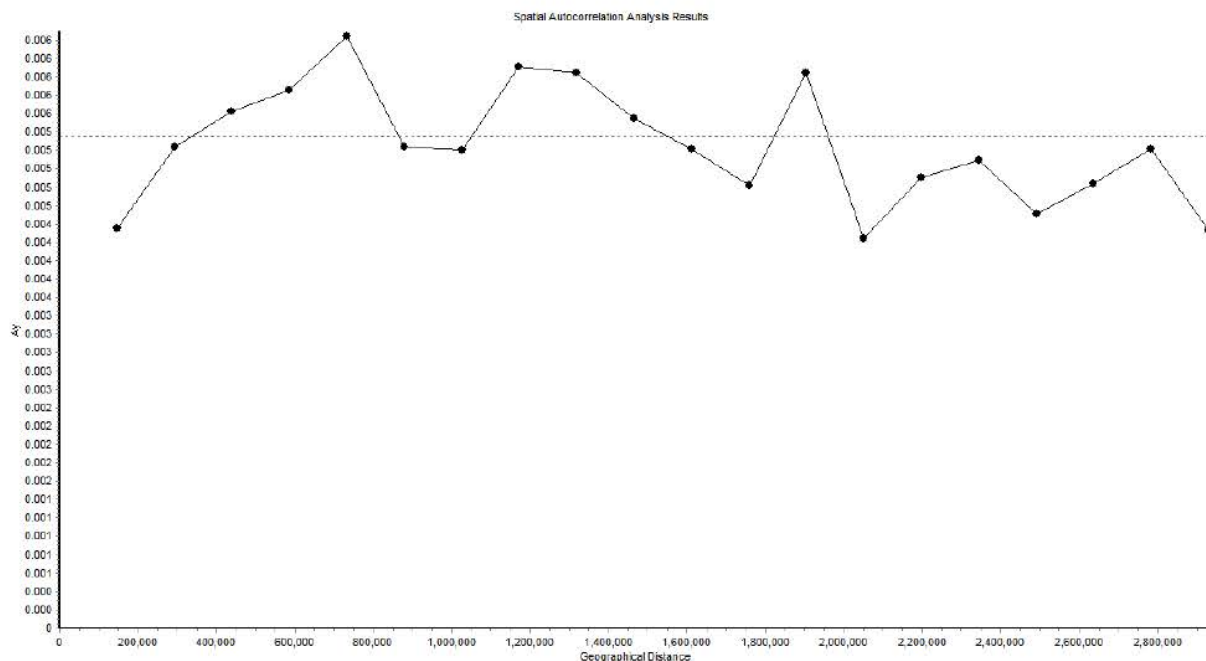


Figure 5: Result of the Spatial Autocorrelation Analysis of the North American *Gulo gulo* Control Region haplotypes found in North America. This analysis was performed using $z = 20$ distance classes. Similar results were obtained using $z = 20$ and $z = 30$ (not reported). The horizontal dotted line indicates the average value of A_y . Geographic distance is plotted in the x axis and the y axis the A_y values.

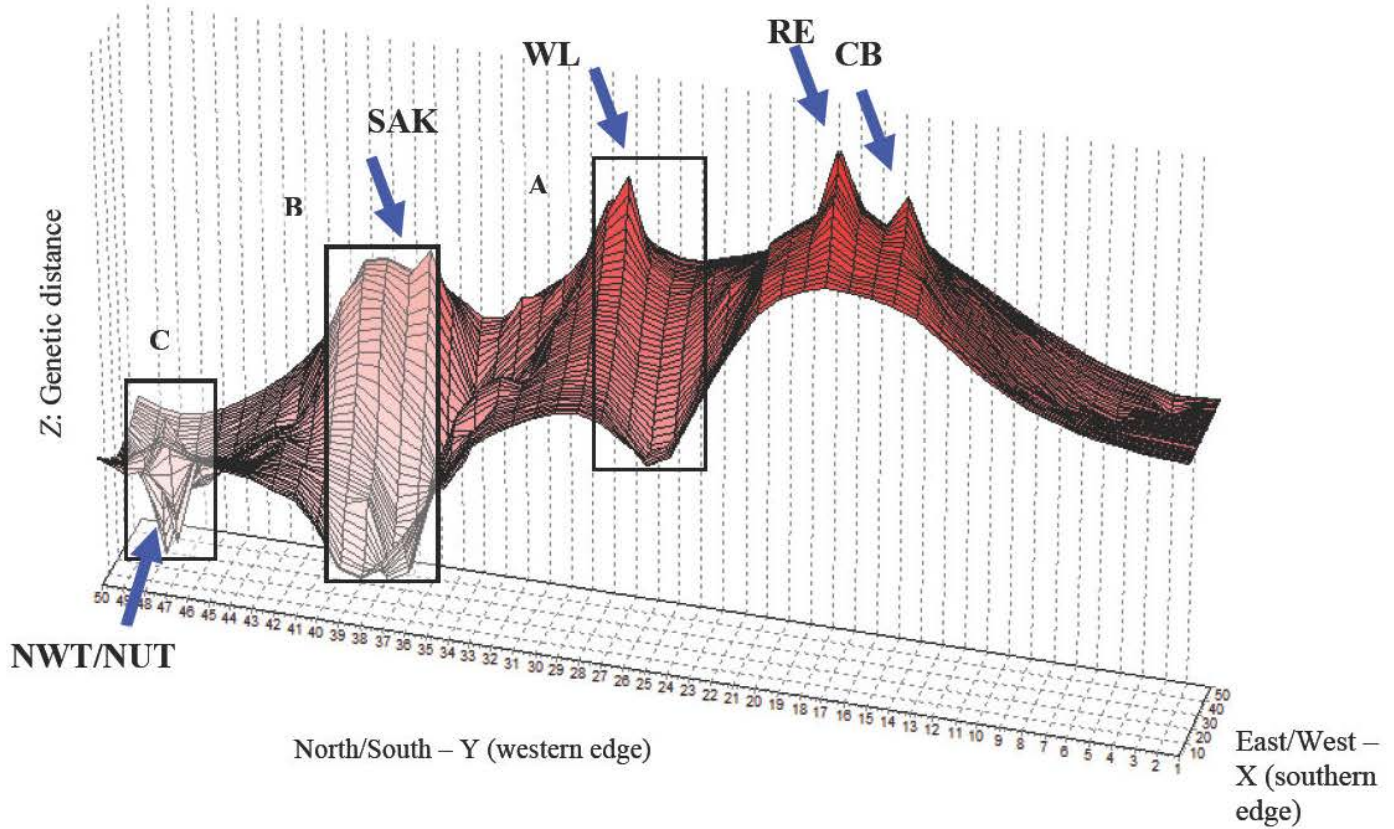


Figure 6: Genetic Landscape Shape interpolation using 50 x 50 grid and a distance weighting parameter of 0.1 (better at showing the general trend across such a broad sampling area). The x and y axis correspond to the GPS coordinates at each locality (UTM). The z axis represents genetic distances (Nei's unbiased D). Blue arrows indicate high genetic discontinuities for that particular population. The black boxes (A-C) represent W-E genetic discontinuities.

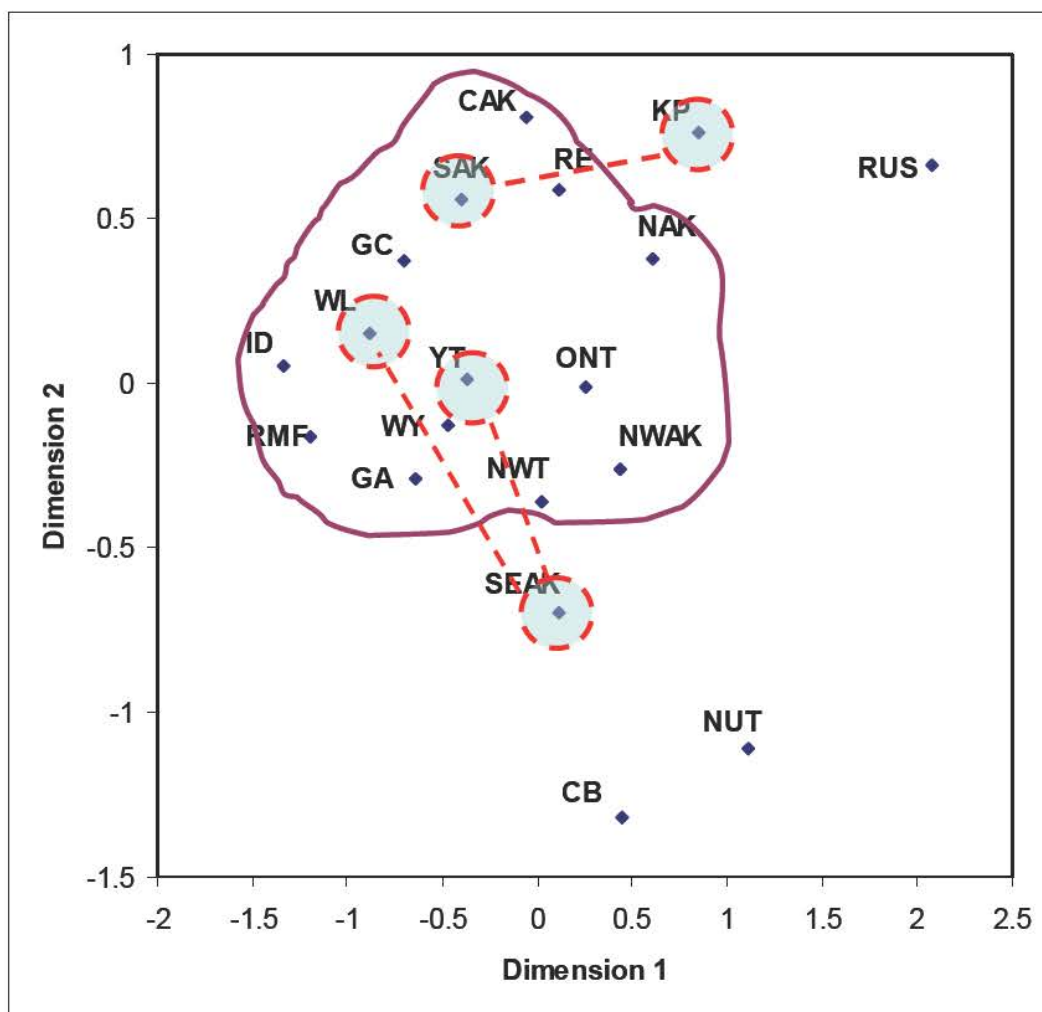


Figure 7: Multi-Dimensional Scaling plot obtained from the F_{ST} table (Table 3). Blue dots represent the different sampling localities. The abbreviations mean: WAK (Western Alaska), NAK (Northern Alaska), CAK (Central Alaska), SAK (Southern Alaska), KP (Kenai Peninsula), SE AK (Southeast Alaska), NWT (Northwest territories), NUT (Nunavut), ONT (Ontario), WL (British Columbia), GC (Alberta), RE (Revelstoke), RMF (Montana 1), GA (Montana2), CB (Montana 3), ID (Idaho), WY (Wyoming), RUS (Russia). Dashed circles and lines indicate the distance between southeast Alaska and Kenai Peninsula and their correspondent inland populations.

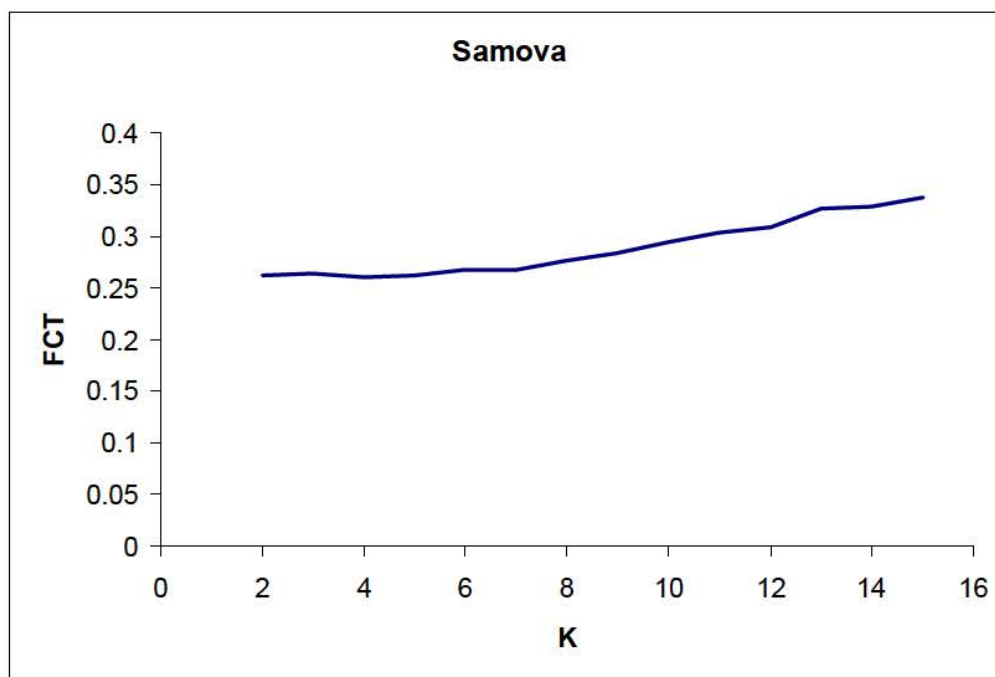


Figure 8: Plot of the F_{CT} values vs K values. F_{CT} values increase monotonically as K increases.

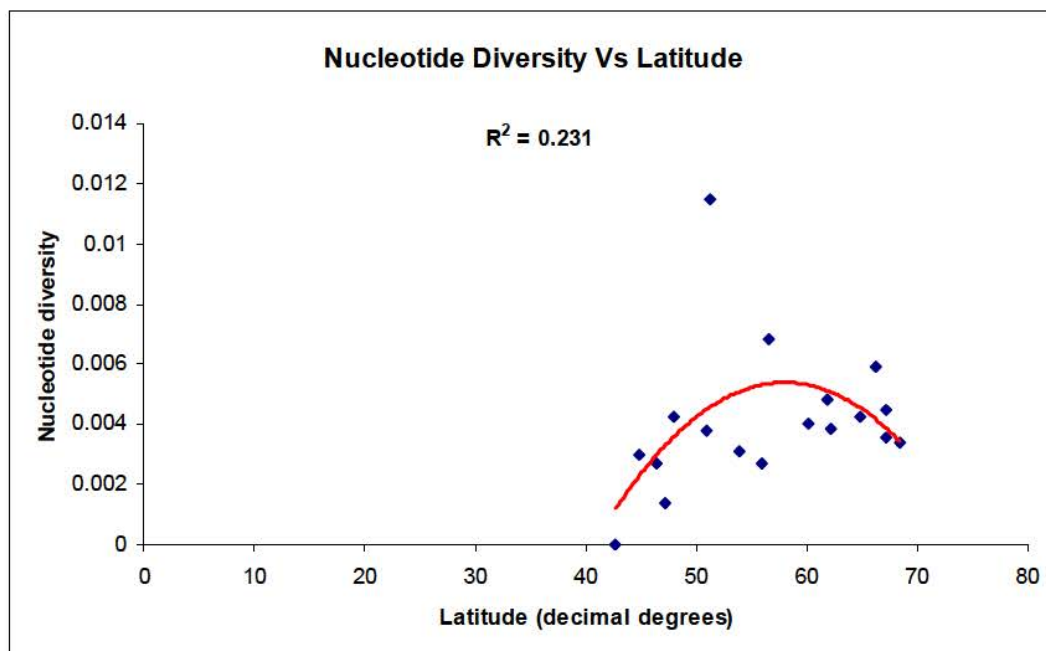
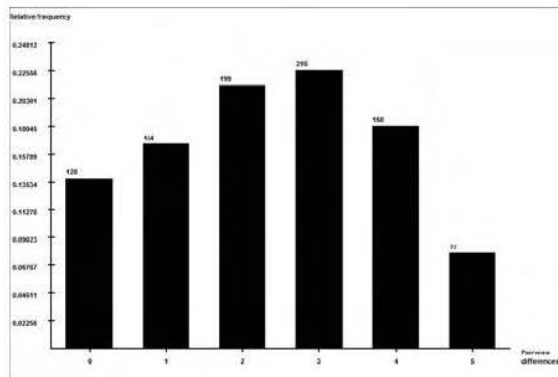


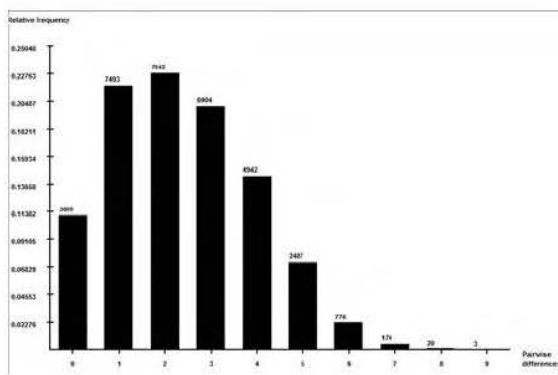
Figure 9: Nucleotide diversity versus latitude. The best fit was a second order polynomial regression ($R^2 = 0.231$) while a linear regression plot had a worse fit ($R^2 = 0.05$). Nucleotide diversity was generally higher at mid-latitude sites.

Beringia Refugia



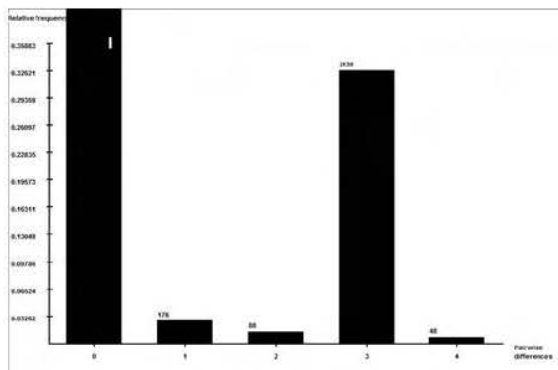
Raggedness statistic r : 0.0642

Glaciated Areas



Raggedness statistic r : 0.0402

Southern Refugia



Raggedness statistic r : 0.0486

Figure 10: Mismatch distribution for the 3 population groupings. The Southern Refugia and the Beringia Refugia show population stability ($r > 0.05$). The Glaciated Areas show population expansion ($r < 0.05$).

Tables

Table 1: Population, specimens, individual museum catalog number, GenBank numbers. AF: Alaska Frozen Tissue Collection. Numbers in bold represent new specimens analyzed for this study (GenBank accession number). * indicate GenBank numbers from previous studies. + indicate sampling localities used for population level analyses.

Population	Sample size	Individual Identification number
Southern Alaska (SAK) ⁺	42	EU812421, 344, 347, 349, 353, 355, 360, 365, 367, 371, 377, 378, 381, 387, 389, 391, 394, 395, 397, 404, 405, 410, 418, 419, 422, 429, 430, 431, 434, 437, 440 , AF1355, 5208, 24800, 01-02, 39937-38, 44, 46, 49, 51
Central Alaska (CAK) ⁺	9	EU812373, 396, 411 , AF 11783, 84, 11801, 27577, 30412, 30422
Idaho (ID) ⁺	15	No museum specimens recorded
Alberta (GC) ⁺	17	No museum specimens recorded
Montana 1(RMF) ⁺	44	No museum specimens recorded
Montana 2 (GA) ⁺	19	No museum specimens recorded
Montana 3 (CB) ⁺	25	No museum specimens recorded
British Columbia 1 (WL) ⁺	24	19 with no museum specimens recorded,
NK140449-50, 55-57		
Wyoming (WY) ⁺	13	No museum specimens recorded
Revelstoke (RE) ⁺	16	No museum specimens recorded
Yukon Territories (YT) ⁺	23	EU812374, 350-352, 356, 357, 363, 364, 382, 388, 400, 403, 409, 414, 424, 428, 441-444, 447, 451, 453
Southeast Alaska (SE AK) ⁺	31	EU812399, 346, 348, 358, 359, 361, 379, 380, 398, 401, 407, 412, 413, 416, 426, 432, 435, 436, 446 , AF 15901-02, 16071, 20010, 51817-22, 51874-75
Ontario (Ont) ⁺	8	EU812445, 354, 362, 439, 448, 449, 450, 452
Kenai Peninsula (KP) ⁺	25	EU812406, 390, 399, 438
Northern Alaska (NAK) ⁺	11	EU812415 , AF354, 4033, 36, 42, 48, 17989, 24882, 27234, 27137, 34266
Northwestern Alaska (NWAK) ⁺	24	EU812345 , AF32004, 45482, 45909, 911, 914-915, 917, 919, 921, 944, 948, 950, 953, 955, 961, 966, 967, 970, 974, 986-988, 47755
Nunavut territories (UT) ⁺	51	EU812369, 386, 417 , AF 55314-16, 20, 22, 24, 25-26, 93-99, 55400-03, 08, 210105-32*
Northwest Territories (NWT) ⁺	27	EU812392, 372, 376, 383, 384, 385, 393, 402, 420, 425 , AF210090-210104*, AF55409, 55410
Eurasia (RUS)	5	AF52357-8, 52388, 52360-1
California (CAL)	7	AY880313*, AY880314*
Mongolia (MON)	1	AY880315*
Scandinavia (SCN)	8	AF 245496*

Table 2: F_{ST} population pairwise comparison. Numbers in bold represent significant values at the 0.05 level, bold represent significant values at the 0.01 level.

	RM	GA	CB	WY	ID	YT	SEAK	ONT	GC	WL	RE	NAK	KP	NWAK	SAK	UT	NVT
1 RM																	
2 GA	0.02042																
3 CB	0.55819*	0.34573*															
4 WY	0.00258	-0.05009	0.33494*														
5 ID	0.01968	0.12023	0.60723*	0.12088													
6 YT	0.08144*	0.03966	0.37896*	-0.00456	0.10952												
7 SEAK	0.3455*	0.19427*	0.11363	0.17238*	0.34473*	0.21594*											
8 ONT	0.3144*	0.15695	0.22368	0.07507	0.25466*	0.11349*	0.13785										
9 GC	0.24838*	0.17117*	0.43479*	0.1361*	0.29505*	0.1363*	0.28907*	0.19216									
10 WL	0.0925	0.09091*	0.47149*	0.06535	0.10185*	0.10037*	0.294*	0.22005*	0.00801								
11 RE	0.32023*	0.23128*	0.45857*	0.14106	0.33564*	0.16242*	0.31237*	0.12083	0.16144*	0.18164							
12 NAK	0.4489*	0.31332*	0.47467*	0.2582*	0.52928*	0.18654*	0.31345*	0.16513*	0.34181	0.35007	0.29375*						
13 KP	0.5109*	0.39469*	0.47812*	0.32349*	0.51266*	0.29887*	0.34703*	0.21298*	0.40521*	0.43556*	0.22902*	0.36631*					
14 NWAK	0.35577*	0.20398*	0.20867*	0.13914*	0.35905*	0.16134*	0.10246*	0.06413	0.26861*	0.29605*	0.14894*	0.22862*	0.17973*				
15 SAK	0.14403*	0.12179*	0.4196*	0.08653*	0.11936*	0.11417*	0.26413	0.19502*	0.19746	0.14442*	0.20897*	0.2536*	0.23504*	0.25943*			
16 UT	0.5732*	0.4381*	0.25029*	0.40581*	0.60096*	0.41903*	0.2796*	0.31216*	0.48951*	0.52905*	0.42031*	0.48218*	0.3809*	0.15702*	0.48416*		
17 NVT	0.24985*	0.10014	0.19224	0.06648	0.29919*	0.08017	0.13919*	0.1081*	0.20535*	0.22751*	0.21945*	0.16315	0.27197*	0.07161	0.21197*	0.21273*	
18 CAK	0.34838*	0.24261	0.47295*	0.17314	0.3951*	0.17023*	0.28092	0.12352	0.28098	0.25895*	0.19146*	0.27261*	0.12693	0.23119*	-0.01836	0.49668*	0.23485*

Table 3: Nucleotide diversity (π), haplotype diversity (h), sample size (n), number of haplotypes for each sampling locality, number of polymorphic sites, number of private alleles (alleles found in only 1 population), and frequency of private alleles (# Private alleles/ n).

Population	π	h	n	# of Haplotypes	# Polymorphic Sites	# Private Alleles	Frequency Private Allele
YT	0.003824 +/- 0.002710	0.7470 +/- 0.0822	23	8	10	2	0.087
SEAK	0.006802 +/- 0.004192	0.7075 +/- 0.0545	31	7	9	2	0.064
ONT	0.011537 +/- 0.008602	0.8571 +/- 0.1083	8	5	15	4	0.375
ID	0.000000 +/- 0.000000	0.0000 +/- 0.0000	15	1	0	N/A	N/A
WY	0.003004 +/- 0.002358	0.4103 +/- 0.1539	13	3	4	N/A	N/A
GC	0.003094 +/- 0.002364	0.7059 +/- 0.0782	17	4	3	N/A	N/A
WL	0.002725 +/- 0.002120	0.6413 +/- 0.0807	24	6	6	2	0.083
RE	0.003802 +/- 0.002758	0.7917 +/- 0.0451	16	4	3	1	0.187
RMF	0.001383 +/- 0.001326	0.1691 +/- 0.0711	44	2	3	N/A	N/A
GA	0.002725 +/- 0.002116	0.3333 +/- 0.0978	25	2	3	N/A	N/A
CB	0.004270 +/- 0.002975	0.5029 +/- 0.1128	19	3	4	N/A	N/A
NAK	0.003378 +/- 0.002608	0.6727 +/- 0.1232	11	4	3	N/A	N/A
KP	0.003989 +/- 0.002790	0.6600 +/- 0.0513	25	3	3	N/A	0.24
NWAK	0.005933 +/- 0.003795	0.7391 +/- 0.0454	24	5	6	N/A	N/A
SAK	0.004791 +/- 0.003150	0.7503 +/- 0.0469	42	10	12	6	0.238
NUT	0.003582 +/- 0.002520	0.8047 +/- 0.0283	51	7	5	N/A	N/A
NWT	0.004456 +/- 0.003021	0.8490 +/- 0.0379	27	8	6	1	0.037
CAK	0.004250 +/- 0.003166	0.6944 +/- 0.1470	9	4	4	N/A	N/A

Table 4: Amova table. The majority of the variance was found within populations.

Source variation	d.f.	Sum of squares	Components	Percentage variation
Among groups	1	12.072	0.01917	1.89
Among populations	16	114.204	0.281	27.65
Within populations	406	290.788	0.71623	70.47
Total	423	417.064	1.0164	

Table 5: Neutrality tests (Tajima and Fu), neither of the reported values was significant (P-value > 0.05) and population size fluctuation analysis (g, bold numbers indicated expanding populations).

	g	SD g	Tajima	Fu
CAK	1252.9	311.5	0.231	'-0.133
CB	-70.6	308.1	0.8	2.344
GA	-78.7	416.6	0.561	3.178
GC	695.5	393.1	0.781	0.092
KP	228.2	298.1	2.08	2.503
NAK	1461.2	494.1	0.708	'-0.29
NWAK	69.2	214.2	0.968	1.23
NWT	1568.9	357.6	0.164	'-2.215
Ont	274.1	100.1	'-0.327	0.85
RE	491.5	339.2	1.56339	0.49
RMF	1687.7	532.5	'-0.486	1.89
SAK	274.6	78.5	'-1.146	'-2.88
SEAK	34.1	175.3	0.671	0.32
NUT	1046.6	120.1	0.395	'-1.031
WL	2218.6	521.8	'-0.913	'-1.96
WY	1482.6	480.1	'-0.478	0.98
YT	2626.6	250.2	-1.25	-3.16
ID	N/A	N/A	N/A	N/A

Box 1: Details of Population Expansion

The genetic consequences of population expansion following glacial recession are considered by comparing populations from regions with different glacial histories. If postglacial colonization took place as a series of successive founder effects (Hewitt 1996), populations in regions that were recently glaciated are expected to (i) have lower genetic diversity than populations from non-glaciated areas, and (ii) exhibit the genetic signature of population expansion from low diversity founder populations. Populations from nonglaciated areas are not expected to show significant population expansion (Miller et al. 2006).

a) Single source recolonization: Populations in the north show signs of population stability while populations in mid and low latitudes should show signs of expansion. We expect to see more genetic diversity in the north than in the south.

b) Double source recolonization: Populations that persisted in the southern and northern refugia should show signs of population stability while populations in mid latitudes should show signs of population expansion. When populations recolonized from both refugia, they met in mid latitudes. We expect to see high levels of diversity in mid latitudes due to the admixture of genetic variants that originated in either high and low latitude refugia.

c) Multiple source recolonization: Wolverine populations survived in multiple refugia (e.g. Beringia, High Arctic, Eastern, and Coastal Refugia) during the LGM thus showing signs of population stability. As glaciers retreated wolverine populations recolonized the previously glaciated areas thus creating multiple overlapping genetic signals in mid latitudes. We expect to see more genetic diversity in mid latitudes.

Signs of Population Expansion:

Tajima	Significant (P-value < 0.05) negative values of D
Fu	Significant (P-value < 0.05) negative values of F_s
g parameter	Positive values that do not include 0 (+/- 3SD)
Nucleotide diversity	Low values
Haplotype diversity	High values
MJN/ Tree	Star-like phylogeny
Mismatch distribution	Unimodal distribution
Raggedness index	r values below 0.05

Signs of Population Stability:

Tajima	Positive values of D
Fu	Positive values of F_s
g parameter	Values that include 0 (+/- 3SD)
Nucleotide diversity	High values
Haplotype diversity	High values
MJN/ Tree	Non star-like phylogeny
Mismatch distribution	Multimodal distribution
Raggedness index	r values above 0.05

Appendix 1 a: Variable nucleotide positions for the 318 bp Control Region fragment.
Insertion/Deletion events are indicated as (-).

Haplotype	Variable Nucleotide Positions																												
	8	10	18	26	29	47	51	56	57	59	60	65	83	111	123	124	125	128	147	153	178	185	200	201	206	209	215	288	293
1 (A)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
2	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	A	T	C	T	G	G	A	T
3	G	A	C	C	A	T	C	A	T	C	T	—	A	T	C	—	T	G	T	C	G	G	T	C	T	G	G	A	T
4	G	A	C	C	A	T	C	A	C	C	C	—	A	T	C	—	T	G	C	C	A	G	T	C	T	G	G	A	T
5	G	A	G	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
6 (F)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	G	T	C	T	G	G	A	T
7	G	A	G	T	A	T	C	A	T	C	C	—	A	T	C	—	T	G	T	C	G	G	T	C	T	G	G	A	T
8 (L)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	T	T	G	G	A	T
9 (G)	G	A	C	C	A	T	C	A	T	C	T	—	A	T	—	—	T	G	T	C	A	A	T	C	T	G	G	A	T
10 (C)	G	A	C	C	A	T	C	A	T	C	T	—	A	T	—	—	T	G	T	C	A	G	T	C	T	G	G	A	T
11	G	A	C	C	A	T	C	A	T	C	T	—	A	T	C	—	T	G	T	C	A	G	T	C	T	G	G	A	T
12 (B)	G	A	C	C	A	T	C	A	C	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
13 (E)	G	A	C	C	A	T	C	A	T	C	C	—	A	C	—	—	T	G	T	C	A	G	T	C	T	G	G	A	T
14	G	A	C	C	A	T	C	A	T	C	T	—	A	T	C	—	T	G	T	C	G	G	T	C	T	G	G	A	G
15	G	A	—	C	A	T	C	A	T	C	T	—	A	T	C	—	T	G	T	C	G	G	T	C	T	G	G	A	T
16	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	A	A	T
17	G	A	C	C	A	T	C	A	T	C	C	A	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
18	G	A	C	C	A	T	C	A	T	C	T	—	A	T	—	—	T	G	T	C	G	G	T	C	T	A	A	A	T
19	G	A	C	C	A	T	C	A	T	T	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
20	G	A	G	T	A	T	C	A	T	C	C	A	A	T	C	—	—	A	T	C	A	G	T	C	T	G	G	A	T
21	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
22 (H)	G	A	C	C	A	C	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	A	C	C	C	G	G	A	T
23 (I)	G	A	C	C	A	T	C	A	T	C	T	—	A	T	C	C	T	G	T	C	A	G	T	C	T	G	G	A	T
24	G	A	C	C	—	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
25	G	A	G	C	A	T	G	A	T	C	C	—	A	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
26 (E)	G	A	C	C	A	T	C	A	C	C	C	—	A	T	—	—	T	G	T	C	A	G	T	C	T	G	G	A	T
27 (D)	G	A	A	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	G	T	C	T	G	G	A	T
28 (M)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	G	G	T	C	T	G	G	A	T
29 (N)	G	A	C	C	A	T	C	A	T	C	C	—	G	T	—	—	T	G	C	C	A	G	T	C	T	G	G	A	T
30 (O)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	C	C	A	A	T	C	T	G	G	A	T
31 (Mong/L)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	A	C	T	T	G	G	A	T
32 (M)	G	A	C	C	A	T	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	A	C	C	T	G	G	A	T
33 (Cal 1)	G	A	C	C	A	C	C	A	T	C	C	—	A	T	—	—	T	G	T	C	A	A	C	T	T	G	G	A	T
34 (Cal 2)	G	A	C	C	A	C	C	A	T	C	C	—	A	T	—	—	T	G	T	T	A	A	C	T	T	G	G	A	T
35 (Scn)	A	T	C	C	A	T	C	—	T	C	C	—	A	T	—	—	T	G	T	C	A	A	C	C	T	G	G	G	T

Appendix 1 b: Haplotypes sorted by sampling locality for the 318 bp Control Region fragment.

Haplotype	UT	SEAK	KP	Ber Ref	ONT	South Ref	SAK	YT	SWCA	NWT	MON	SCN	RUS	CALI
1	3	14	7	13	3	89	30	11	22	8	0	0	0	0
2	4	0	12	12	0	1	2	0	5	0	0	0	2	0
3	1	13	0	6	0	0	0	1	0	0	0	0	0	0
4	0	0	0	0	0	0	2	0	0	0	0	0	0	0
5	0	0	0	0	0	0	1	0	0	0	0	0	0	0
6	7	0	6	4	0	0	1	4	2	5	0	0	0	0
7	0	0	0	0	0	0	2	0	0	0	0	0	0	0
8	0	0	0	0	0	2	0	0	19	0	0	0	0	0
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32	0	0	0	0	0	0	0	0	0	0	0	0	1	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	6
34	0	0	0	0	0	0	0	0	0	0	0	0	0	1
35	0	0	0	0	0	0	0	0	0	0	0	8	0	0

Appendix C. Manuscript submitted to *Journal of Wildlife Management* on 2 February 2010 describing a field technique for identifying wolverines using an integrated system of motion-detection cameras and hair-snags and tested on wolverines on the mainland of Southeast Alaska near Petersburg in 2009.

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8 RH: Magoun et al. • Wolverine Identification

9 **Wolverine Identification — Integrating Motion-detection Cameras and Hair Snags**

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22 **ABSTRACT** Use of motion-detection cameras and hair snags to monitor wildlife has grown
23 substantially with advancements in these technologies, particularly for rare and elusive species
24 that are difficult or expensive to capture. Wolverines have a pattern of light pelage on their throat
25 and chest that is unique among individuals and does not change over time. We developed an

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integrated system for photographing the ventral surface of a wolverine while simultaneously obtaining hair samples for microsatellite DNA genotyping. The system allowed us to match the hair sample to the photograph. Using the photographs, we also identified the sex of the individuals. We tested this system on wild wolverines in Southeast Alaska in 2009 and validated the identity and sex of individuals in the photographs with genotypes provided by the DNA analysis. There was a 100% match between the photographic and DNA data from 35 integrated collections of photographs and hair from 13 individual wolverines (8 M, 5 F) captured at 18 stations. The system we developed is not only useful for documenting individual identity and sex of wolverines captured in photographs, but also for reducing the cost of DNA analysis and providing information on lactation by females in photographs, familial relationships from DNA, and other population and behavioral data. We encourage managers to continue to develop and refine the technique for maximum yield of information from cameras and hair snags.

KEY WORDS DNA, microsatellite genotyping, motion-detection cameras, *Gulo gulo*, hair snagging, identification, photographs, Southeast Alaska, wolverine.

The Journal of Wildlife Management: 00(0): 000–000, 2010

Wolverines range widely, occur at low densities, and occupy habitats that are remote and often difficult to access (Copeland and Whitman 2003). For these reasons, ecological studies of wolverines have been expensive and labor intensive, relying primarily on radiotelemetry for much of current knowledge of wolverine spacing patterns and population parameters (Persson et al. 2009). Use of motion-detection cameras and microsatellite genotyping to monitor wolverines and other cryptic carnivores is becoming increasingly common with recent advances in these detection techniques (Lofroth and Krebs 2007, Koen et al. 2008, Balme et al. 2009, Fisher et al. 2009). Published evaluations of noninvasive techniques for monitoring wildlife focus on

effectiveness and cost comparisons between methodologies, and recommendations for integrating methods emphasize multi-species monitoring (Koen et al. 2008, Long et al. 2008, Balme et al. 2009). We developed an integrated system of cameras and hair snags, targeted specifically to wolverines, to maximize the amount of information about wolverine populations that can be gained from using a combination of noninvasive techniques. Wolverines have a pattern of light pelage on their throat and chest (ventral pattern) that is unique among individuals (Fig. 1) and does not change appreciably over time (A. J. Magoun, WRAM, unpublished data). Photographs of the ventral surface of a wolverine can be used to identify not only the individual but also its sex (Fig. 2). With the integrated system we designed, it is possible to photograph the ventral pattern and the abdominal area of a wolverine while simultaneously collecting hair from the animal. The next step in development of this technique was to test the system on a wild population of wolverines, which is the subject of this paper. Specifically, our objectives were to 1) construct an array of camera and hair snag (C&H) stations in forested habitat in Southeast Alaska and test the system's effectiveness at simultaneously capturing photographs and hair of wild wolverines, 2) validate our ability to determine identity (ID) and sex from photographs by comparing the photographic data on ID and sex with that from DNA, and 3) recommend further refinements in the system and encourage researchers and managers to test the system in different wolverine populations.

STUDY AREA

The study area comprised 2,140 km² of coastal tidelands, temperate rainforest, muskeg, alpine habitat, and glaciers in the Tongass National Forest on the mainland of Southeast Alaska near Petersburg (56° 48.67602' N, -132° 57.07452' W). Boundaries of the study area were Port Houghton on the north, LeConte Bay on the south, Frederick Sound on the west, and the Stikine

Icefield on the east. Elevations ranged from sea level to 2,164 m. Most forested habitat occurred within 20–50 km of the coast and comprised Sitka spruce (*Picea sitchensis*) or western hemlock (*Tsuga heterophylla*) on productive forest sites and mountain hemlock (*Tsuga mertensiana*), Alaska-yellow cedar (*Chamaecyparis nootkatensis*), or western red cedar (*Thuja plicata*) on poorly drained sites. Temperatures at sea level during the study ranged from -4°C to 7°C but were considerably colder at higher elevations, although rarely falling below -23°C . Snowfall during the study was very heavy, reaching depths $>4\text{ m}$ at higher elevations. Human inhabitants and developments were clustered near Point Agassiz where there was a local network of old logging roads. Access for trapping was either along this road system with snowmachines and 4-wheelers or along the coast with boats. The average annual harvest of wolverines in the study area in 2000–2009 was 2 (range 1–6) with males comprising 67% of the total harvest for those years. Trappers limited their efforts to take wolverines in 2007–2009 in cooperation with this study.

METHODS

From February–June 2009, we set up 29 C&H stations in forest stands from sea level to 670 m above sea level. We accessed these sites using boats, snowmachines, 4-wheelers, or helicopters. At each station we attached a horizontal, 120-cm-long run pole to a tree with the proximal end at least 1 m above bare ground or snow and with the distal end pointed toward another tree approximately 3–4 m away (Fig. 3). We fabricated the run pole from 2 x 4 dimensional lumber (38 mm x 89 mm) or used a natural log ($\sim 15\text{ cm}$ diam) found on site. To attach the hair-snag structure (described below) to the pole, we used 2 short pieces of 2 x 4 dimensional lumber, one that was 35.6 cm (14 inches) long and one that was 40.6 cm (16 inches) long, and attached the shorter piece with screws to the run pole so that it was perpendicular to the pole, flush with the

distal end of the pole, and centered on the pole. The longer crosspiece was positioned similarly but was 22 cm from the distal end of the run pole (Fig. 3).

We used square rods (~ 2.5 cm) of ultra high molecular weight (UHMW) polyethylene plastic (Plastic Supply Inc., Tacoma, WA) and steel alligator clips with 7.9-mm jaw opening (Mueller Electric Company, Akron, OH) to construct hair-snag structures, which we mounted at the distal end of the run poles (Fig. 3). The support structure consisted of 1) 2 vertical, 76-cm-long pieces of UHMW plastic (uprights), each screwed at their base to opposite ends of the shorter crosspiece on the run pole; 2) 2 similar pieces of UHMW mounted diagonally for bracing the 2 uprights (braces) with one end of each piece bolted to the top of the UHMW uprights and the other screwed into the end of the longer wooden crosspiece on the run pole; and 3) a 46-cm-long, 6.4-mm-diam ($\frac{1}{4}$ inch) threaded steel rod that spanned the distance between the uprights ~2.5 cm from the top of the structure (crossbar). We used 2 additional pieces of UHMW ~30–40 cm long (snag posts) on which we mounted 6–8 alligator clips (Model #BU-60PR2) (Fig 4). We inserted the base ends of the clips into 6.4-mm-diam ($\frac{1}{4}$ inch) holes drilled into the snag posts and, tapping on the point of the jaw lightly with a hammer, firmly seated the base clips into the holes. We screwed the snag posts vertically into the proximal edge of the longer crosspiece, one on each side of the run pole separated by ~25 cm (Fig. 4). When the snagging device was set, each of the base clips held open a trigger clip (Model #BU-60). We attached the trigger clips to Henssger simplex snaps® (Henssger Hardware Corporation, Glens Falls, NY) with lanyards of Steelon® nylon-coated wire (BD45BL) (Berkley®, Spirit Lake, IA) and Berkley connector sleeves (B3) and secured the clips to the snag posts by snapping the spring clips onto small eye screws inserted into the snag posts (Fig. 4). The springs in the base clips were at least as strong as those in the trigger clips so that the base clips held the trigger clips open to the widest extent

possible. When dislodged by an animal, the trigger clips would dangle at the end of the lanyard, preventing another animal from depositing hair in the clip (Fig. 5).

We attached a digital, motion-detection camera (Trailwatcher®, Monticello, GA) to the tree opposite the run pole so that the camera faced the distal end of the run pole. We set the camera to take photos with the flash on both day and night to provide sharp photographs with no shadows. The camera triggered every time an animal moved in front of the camera, resulting in photos taken ~4 sec apart for 500–700 photos on a 2 gigabyte memory card. For back-up in case of malfunction, battery depletion, or a full memory card, we also attached another Trailwatcher camera or an infrared (no flash) camera (Reconyx®, Holmen, WI) to the tree and set the cameras to take photos over a longer period of time. The Reconyx cameras generally produced poorer quality photos but would operate at temperatures below –23° C. We adjusted the height and position of the cameras so that the identification number on the end of the run pole was centered near the bottom of the photographs. We ran a cable (2-mm-thick wire rope) horizontally between the 2 trees and high enough that the bottom of the bait was ~30–40 cm above the crossbar. Heavier pieces of bait required a higher horizontal cable. For bait, we used parts of deer (*Odocoileus hemionus*), moose (*Alces alces*), black bear (*Ursus americanus*), or beaver (*Castor canadensis*) with a major bone attached. Baits were either road-killed animals or the remnants of animals taken by hunters or trappers. We threaded a piece of cable through a hole drilled through bone in the bait and, using a cable clamp, attached the bait cable to the horizontal cable and adjusted the height and location of the bait above the distal end of the run pole before tightening the cable clamp.

When we visited the C&H stations to collect hair, which we did each time we checked cameras to change batteries and memory cards at 1–4-week intervals, we placed the hair from

each trigger clip (Fig. 4) into a separate small envelope and labeled the envelope with an accession identification number (AIN) that included the date, camera site, and clip position on the snag post (e.g., C10-041809-R7 indicated a sample of hair collected at camera site C10 on 18 Apr 2009 from the seventh clip from the top on the right snag post). We then placed these envelopes into a larger envelope labeled with a collection identification number (CIN) that included the camera site and collection date (e.g., C10-041809). Because we had mounted the alligator clips so they could be seen on the photographs, we could tell which clips were set and which ones were triggered both before and after a visit by a wolverine (Fig. 5), thereby allowing us to determine which individual wolverine was associated with each triggered clip. This integrated method of photographing and collecting hair from wolverines made it possible to link genotypes with the unique ventral patterns of the wolverines photographed at the sites.

We extracted genomic DNA from hair samples using standard protocols for tissues (Dneasy Tissue kit; Qiagen Inc., Hilden, Germany), with overnight incubation in lysis buffer and Proteinase K on a rocker at 60 °C. We examined the hair samples at 9 microsatellite loci used previously with wolverine samples in other studies: *Gg4*, *Gg7*, *Ma2*, *Ma8*, *Tt4* (Davis and Strobeck 1998); *Ggu101*, *Ggu216*, *Ggu234* and *Ggu238* (Duffy et al. 1998). We amplified microsatellites using protocols detailed in the respective sources, except we amplified these microsatellites using 0.8 units of Taq polymerase and 45 PCR cycles. The resulting products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology, Lincoln, NE). We accepted data from hair samples as error-free only if the microsatellites produced consistent scores in 3 separate PCR reactions. In addition, we used an SRX/SRY region to determine sex (Hedmark et al. 2004).

To validate the accuracy of ID and sex determined from photographs of the wild wolverines, we compared results from photographs with that from DNA using the following protocol. From photographs with a full ventral pattern, author Magoun determined ID of the wolverines and assigned a name for each individual with a unique ventral pattern. From photographs of the wolverines in a bipedal stance, she identified the sex of the animals. In addition, by examining the photos, Magoun determined which clips were triggered by a particular animal and assigned the AIN of each clip to that animal. She then provided a list of the wolverine names and their sex with the associated CIN and AIN data to author Long prior to DNA analysis of the hairs. Without knowledge of ID and sex of wolverines identified from photographs, the genetics laboratory extracted DNA from the hairs and identified the genotypes and sex of the individuals in each hair collection having suitable DNA. They assigned a code number to each genotype and provided a list of the DNA ID codes, sex, and associated CIN and AIN data to Magoun and Long, who independently compared the data files to their individual results. Using this protocol, we avoided observer bias in the validation process.

RESULTS

In 2009 we collected 188 hair samples in 41 collections at 18 of the 29 (62%) C&H stations. To reduce the cost of DNA analysis, we selected 35 collections for which we had identifiable photos of wolverines and could determine which trigger clips were associated with the photographed animals. We subsampled in each collection when photos showed that only 1 individual had visited the site before the hair was collected or, in the case of a visit by >1 individual, we could see in the photos which wolverine had triggered which clips. If we could not match a wolverine with a particular clip and >1 individual had potentially contributed hair to a single collection, then we analyzed all samples in the collection separately, but we had only one occurrence of >1

individual's DNA represented in a single collection. In this collection, photographs indicated 2 wolverines triggered clips at this C&H station, and we were able to identify the individuals from the photographs and their genotypes from hair collected at the station. We performed DNA extractions on 74 hair samples using 9 variable microsatellite loci and obtained quality DNA for genotyping of 37 of the 74 samples (50%), which represented 28 of the 35 (80%) collections we had selected.

We obtained identifiable photographs of wolverines at 22 of the 29 (76%) stations. Hair from 2 stations was too poor to analyze for DNA. Of 14 different wolverines (8 M, 6 F) identified from photographs and associated with hair collections, we had sufficient DNA to genotype 13 individuals (8 M, 5 F), the DNA quality being too poor to genotype one of the females. For those collections with good quality DNA, there was 100% agreement between the photographic data and the DNA data, both for ID and sex. Although 6 other collections also had poor quality DNA, it was possible to genotype these individuals from other hair collections matched to the respective photographs.

Two of the animals in the photographs wore radiocollars, having been captured in live traps in 2008 as part of another study (A. J. Magoun, unpublished data). Therefore, we based our test of sex identification from photographs on the remaining 11 individuals that we genotyped. Our determination of sex from photographs matched that from DNA in every instance.

Of the 13 individuals identified by both photographs and DNA, we identified 5 from only 1 collection and 8 from 2–6 collections. All individuals identified from multiple collections were identified by both photographs and DNA in the same collections. In addition to the 14 individuals photographed at stations where hair was collected, we identified 4 other individuals from photographs at C&H stations (1 M, 1 F, and 2 of undetermined sex) that could not be

genotyped because either the triggered clips had not snagged hair, the clips were already triggered by another animal, or the wolverines failed to trigger clips. We found no genotype that could not be matched with a photograph.

DISCUSSION

Using photographs of individuals responsible for triggering the alligator clips, we were able to subsample from between and within the hair collections without compromising our ability to identify all individuals that left hair at the C&H stations, an advantage of using alligator clips over barbed wire for snagging hair from wolverines (e.g., Mulders et al. 2007). Moreover, we believe that alligator clips were superior to barbed wire in ease of deployment, reduced risk of injury, and the additional information provided by linking genotypes to individuals identified from photographs. By associating a genotype with a unique ventral pattern, subsequent hair collections can be sorted by individual and would not require DNA analysis when photographs indicate a known individual. Effort can then be concentrated on obtaining hair at stations where photographs indicate wolverines without known genotypes are using the sites, including new individuals that may be offspring of known individuals. For large-scale or multi-year studies, limiting the number of hair samples that must be processed and analyzed for DNA, while ensuring a comprehensive database of individuals in the study, could potentially save thousands of dollars in laboratory costs.

One disadvantage of using alligator clips is that once animals have triggered all the clips, subsequent visits by wolverines before the clips are reset will not yield hair for DNA analysis. We believe this disadvantage is outweighed by eliminating samples that could contain hairs from multiple animals and by the ability to link genotypes to photographs. We were able to genotype 13 of 18 (72%) wolverines photographed at our stations using alligator clips and we believe that

we can improve on this success rate with additional refinements in our hair-snagging technique. No information is available on the success rate of genotyping wolverines visiting stations where hair is collected using barbed wire, although Fisher et al. (2009) reported that evidence from motion-detection cameras indicated that wolverines visited such stations without depositing hair.

We suggest the following precautions when applying the technique to other wolverine populations. In this study, we linked DNA to 5 individuals identified by photographs using a single hair collection each. Whenever possible, at least 2 hair collections should be used to link unique ventral patterns to individual genotypes as a way to cross-validate the linkage. We also strongly recommend that only full ventral patterns (i.e., entire throat and chest) be linked to genotypes to avoid errors in the initial linkage and incorrect associations in subsequent collections of photos or hair. Furthermore, if there is any doubt that a particular alligator clip was triggered by the wolverine in the photograph, then at least one additional hair collection for that individual should be genotyped if available. Loose hairs on the hair-snagging structure should not be used to link a genotype with a particular wolverine, even if no other wolverine was photographed at the site, to prevent mistakes in matching genotypes to ventral patterns. Even if photographs indicate that loose hairs are almost certainly from a particular individual, verification from another collection should still be made before assigning a final genotype to that individual.

Management Implications

Besides providing information on ID, sex, and genotype as in this study, the integrated system of motion-detection cameras and hair snags has the potential to provide data on familial relationships and movement patterns of uncollared individuals based on DNA, survival rates in multi-year studies, lactation in females, centers of activity, population density using capture-

recapture models, and behavioral information such as association of individuals based on the date and time stamp on photographs. In fact, we believe that the full value of the system will not be realized until other researchers and managers apply the technique to their particular conservation and management needs. We encourage modifications of the system to improve the design and to suit different habitat types (e.g., tundra vs. forest) and different wolverine populations (e.g., trapped vs. untrapped) where behavior of wolverines might differ. Finally, the photographs we obtained of marten (*Martes americana*) at our C&H stations suggest that a similar integrated system of cameras and hair snags would work equally well for martens with modifications for the smaller size of this forest carnivore.

Acknowledgments

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309 List of Figures

310 Figure 1. Ventral patterns of 10 different captive wolverines photographed on 15 November
311 2008 with a motion-detection camera at night (photographs provided by D. Pedersen),
312 indicating that even closely-related or similarly-patterned individuals can be identified
313 from photographs. All wolverines in the photographs are males except the female in the
314 lower right-hand corner.

315 Figure 2. Photographs of 3 different wolverines taken with motion-detection cameras at sites in
316 Southeast Alaska showing the abdominal area with evidence of sex for a $M \geq 2$ years old
317 photographed on 11 May 2009 (top left), a non-lactating female photographed on 12 May
318 2009 (top right), and a lactating female photographed on 13 May 2009 (lower left) and
319 again on 26 June 2009 (lower right) showing regression of teat size after weaning.

320 Figure 3. Photographs of structures mounted on the end of run poles designed for snagging hair
321 from wolverines as part of a integrated system of motion-detection cameras and hair-
322 snagging devices with 1) a side view showing the positions of the bait, crossbar, uprights,
323 braces, crosspieces, and snag posts with alligator clips (top) and 2) a front view taken in
324 Southeast Alaska by a Trailwatcher® camera mounted on a tree opposite the run pole
325 (bottom).

326 Figure 4. Close-up photographs from Southeast Alaska in 2009 of hair-snag posts and alligator
327 clips showing snag posts with 5 pairs of clips in each post and all trigger clips set (left),
328 trigger clips dislodged by a wolverine with wolverine hair in one of the clips (upper

329 right), and a trigger clip and lanyard ready to be clipped to an eye screw on a snag post
330 (lower right).

331 Figure 5. Photographs of 2 different wild wolverines in Southeast Alaska by a motion-detection
332 camera as they approached along a run pole toward hair-snag posts with alligator clips on
333 2 Apr 2009 (top) with 7 pairs of clips on the left snag post and 6 pairs of clips on the right
334 snag post all set and on 17 Mar 2009 (bottom) with all clips triggered.

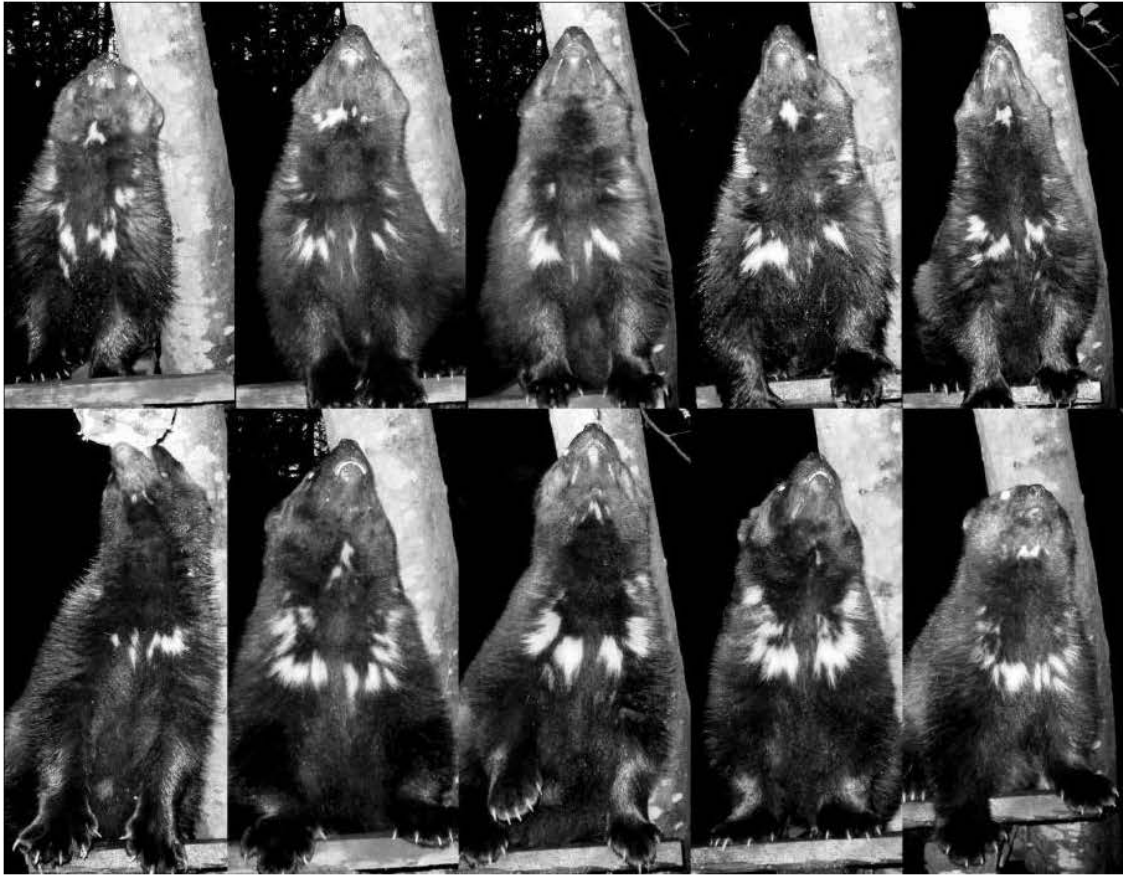


Figure 1

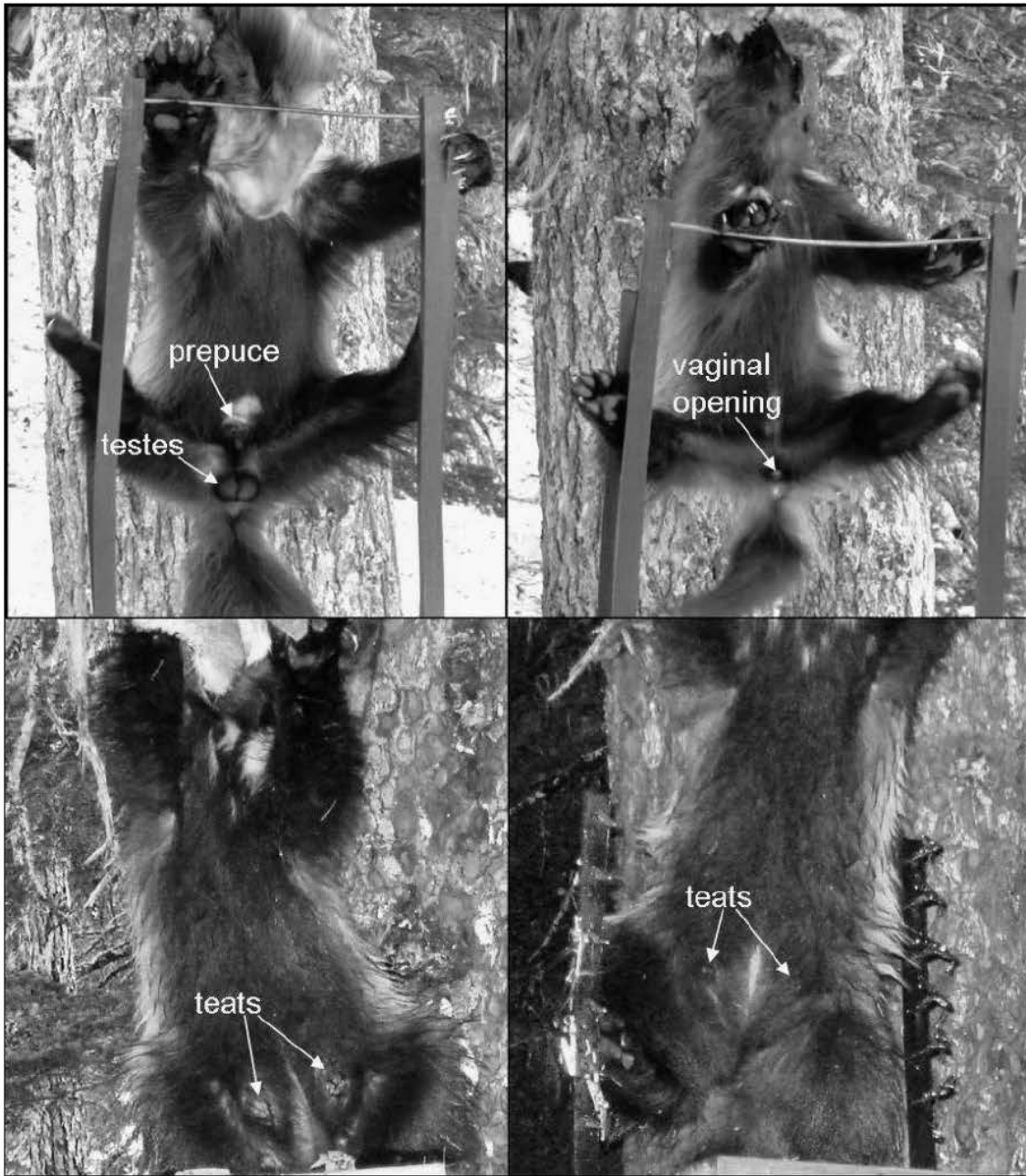


Figure 2

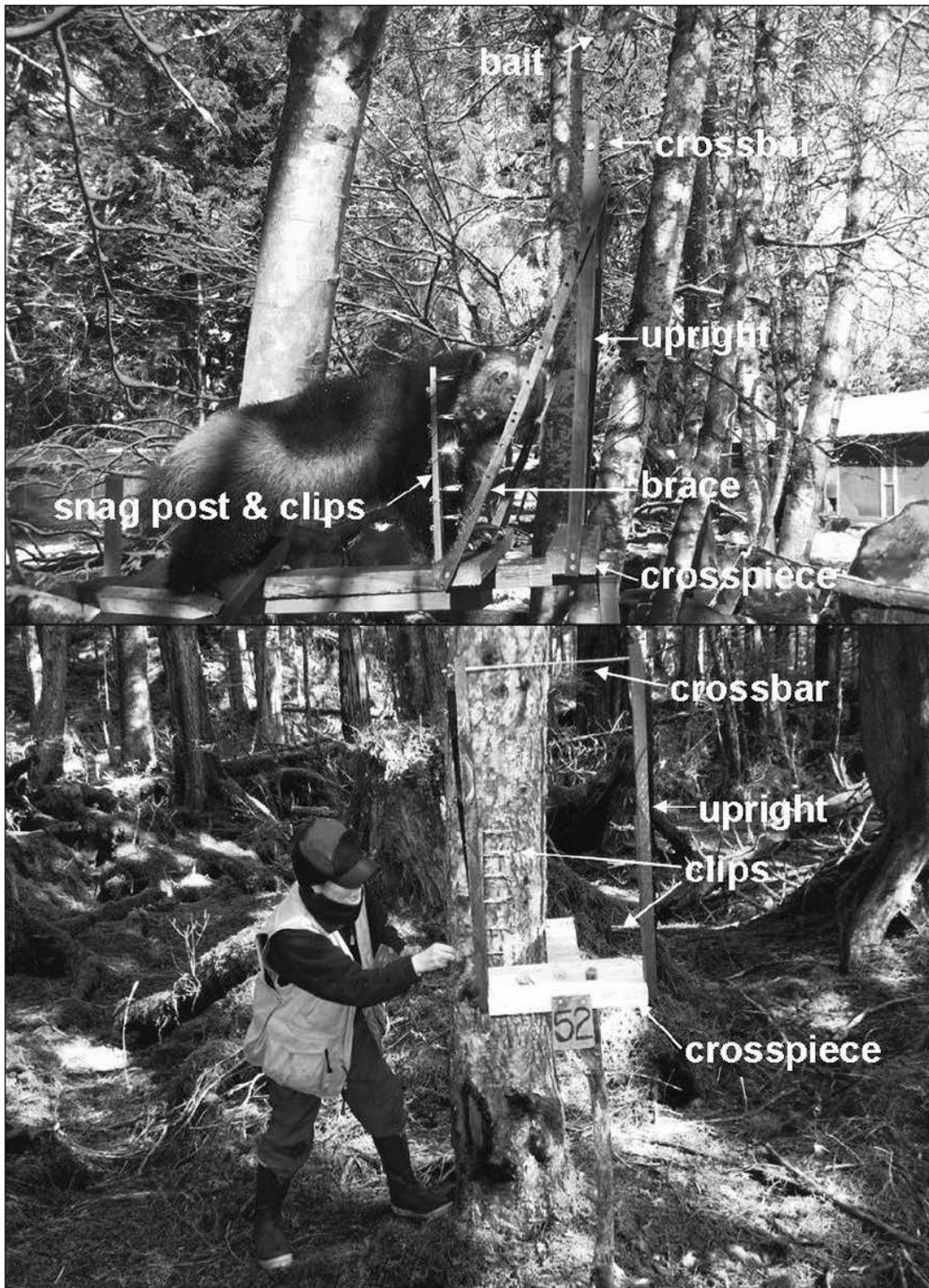


Figure 3



Figure 4

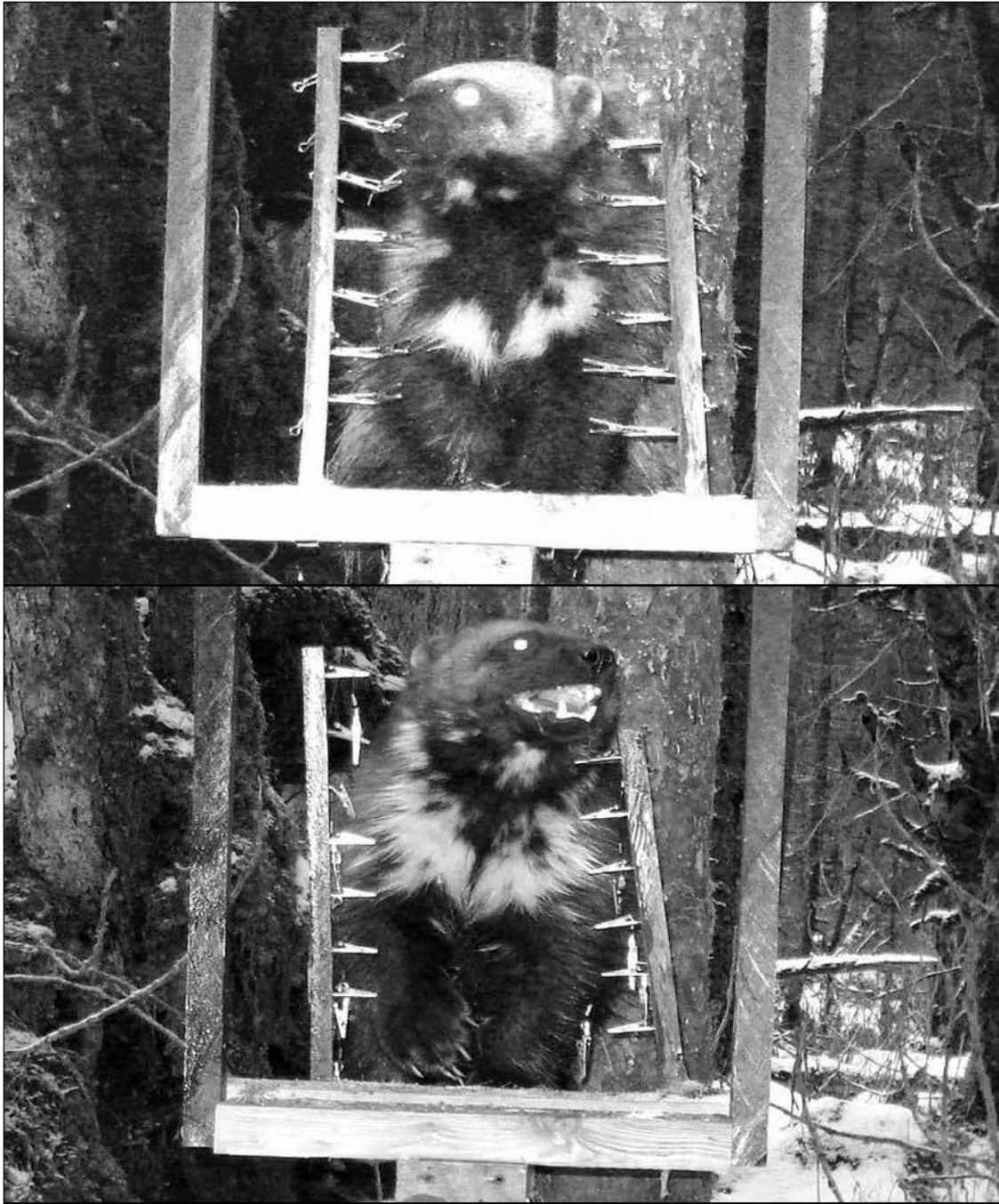


Figure 5